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CELL TYPES AND HISTOLOGIC PATTERNS IN CARCINOMA OF THE LUNG

OBSERVATIONS ON THE SIGNIFICANCE OF TUMORS CONTAINING MORE THAN ONE TYPE OF CELL*

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It is generally agreed that the histologic picture in carcinoma of the lung varies widely, but there is no agreement about the significance of this variability. Willis,[1] for example, includes all morphologic types within a single entity, "carcinoma of the lung," while other authors[2,3] recently have divided their cases into a number of sharply delimited groups that vary from one another in morphologic and in clinical characteristics. In the present work a histologic study has been made of the slides from 234 patients with carcinoma of the lung who were necropsied in the New York Hospital during the past 28 years, together with a review of the clinical histories of the cases. The study shows that in 65 per cent of the cases the tumors were comprised wholly of one or another of the four types of cells later to be designated as large polygonal, small round, epidermoid, or columnar. In the remaining instances the growths contained such an intimate mixture of two or more of these types as to suggest that all four types of cells are derived from a single prototype.

THE FOUR TYPES OF CELLS FOUND IN CARCINOMA OF THE LUNG

Large Polygonal Cells (Type A)

As seen in histologic sections stained with hematoxylin and eosin, the cells of type A are large and ovoid to polygonal. The cytoplasm is abundant. The nuclei usually are vesicular but sometimes deeply stained. The cells themselves and their nuclei usually are somewhat larger than those of bronchial epithelium but in other respects they closely resemble the cells of normal epithelium, as illustrated in Figure

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1. The large polygonal cells are found either in tightly packed masses or arranged around small spaces.

The cellular pattern most frequently found in the entire series of necropsies consisted of large polygonal cells in tightly packed masses. The tumor cells projected into the bronchial lumen in only a few instances. In the bronchial walls the tumor cells regularly formed compact masses or sheets, but in occasional slides the carcinomatous cells were separated by thin strands of fibrous tissue into radiating bundles. The edges of the masses of tumor sometimes were indistinct, their patterns merging with the surrounding tissue; but they were arranged more characteristically in a regular palisade-like layer of ovoid cells. In the pulmonary alveoli, similar compact masses surrounded by palisading cells were the rule, but in many areas the alveoli were packed with loosely arranged elements. The degree of necrosis in the tumor masses varied greatly. Compact masses of cells similar to those just described were found in lymphatic and venous channels near the primary tumor, in the foci of spread of the tumor to such structures as the pericardium, and in numerous metastases. An example of the large polygonal cells in tightly packed masses is seen in Figure 2.

In a smaller number of cases, large polygonal cells in other ways indistinguishable from those described tended to be less tightly packed and, in many areas, to be separated from one another by small spaces that gave no suggestion of well defined lumina. Some of the slides contained areas where the large polygonal cells were found in both of the arrangements noted. Large polygonal cells arranged around small spaces sometimes were found directly adjacent to the bronchial lumen in such a way as to suggest that the carcinoma arose in this pattern, rather than in the more compact form. The structure of the large polygonal cells arranged around small spaces is illustrated in Figures 3 and 4.

Large polygonal cells were found alone or with other types of cells in 72 per cent of all necropsied cases.

Small Round Cells (Type B)

In the present paper the term small round cell is used to indicate a round or slightly ovoid cell somewhat larger than a characteristic adult lymphocyte. It has a dark nucleus about two thirds as wide as it is long and presents scarcely any recognizable cytoplasm when examined in routine paraffin sections stained with hematoxylin and eosin. Sometimes the cells are very tightly packed, sometimes separated from one another by loose areolar tissue. These small cells seem to be those described by Jaffé[4] as "basal" and by Foot[2] as "anaplastic" and to

include some, but by no means all, of the cells entering into the formation of "oat-celled" tumors.[5] Small round cells were found alone or with large polygonal cells in 18 per cent of the necropsies. The structure of a carcinoma composed of cells of this type is shown in Figure 5.

Epidermoid Cells (Type C)

The epidermoid cells tend to be more tightly packed than the large polygonal cells, although this difference is not always definite. When these cells are in close apposition, they are separated by fine or coarse strands of shiny, pale red (with hematoxylin and eosin) material. The nature of this material is not clear but areas may be found where the strands run into foci of undoubted keratinization. No intercellular bridges have been demonstrated. The cytoplasm of these cells is slightly more abundant and tends to be more acidophilic, i.e., redder with hematoxylin and eosin, than that of the large polygonal cells. The nuclei of the epidermoid and large polygonal cells are indistinguishable. Epidermoid cells were found alone or with other cells in 28 per cent of the necropsies. Aggregates of cells of this type include forms with and without pearls. Epidermoid cells without pearls are shown in a regular arrangement in Figure 6, and in a highly irregular pattern in Figure 7. Epidermoid cells with pearls are found much less frequently than are those without pearls. This structure is illustrated in Figure 8.

Columnar Cells Arranged Around Well Defined Lumina (Type D)

Cells of type D form the picture described as "adenocarcinoma" in the original sense of the term since they are arranged in the form of glands. The term is avoided in this paper because of histogenetic implications often associated with it. There may be much or little mucus in the cells and between them. The presence or absence of mucus does not seem to modify the essential nature of these elements or their behavior, as there are gradations from areas with large masses of mucus to others where cells of similar appearance contain no mucus at all. Many alveoli are lined by, or even packed with, similar columnar cells and form the picture described as "terminal bronchiolar" or "alveolar cell carcinoma."[6,7] Type D cells lining the alveoli are very similar morphologically to the columnar variety found closer to the hilum of the lung. In the material under consideration the process was so advanced as to give no clear evidence as to the origin of these cells, but I am in accord with Hutchison's[8] contention that they arise from the mucosa of smaller or larger bronchi. In these advanced cases it does not seem possible to differentiate nodular from diffuse types of terminal bronchiolar tumor.[6,7] Figure 9 gives a good example of columnar cells

around definite lumina. Columnar cells were found alone or with other types in 20 per cent of the necropsies.

Tentative Separation of the Cases into Groups According to the Nature of Their Cellular Content

A number of authors[2,3,9] have divided their cases of carcinoma into four groups depending on the nature of the predominant cell in each case, using a classification essentially similar to that which has been described in the preceding paragraphs. In the present study there were 151 necropsies (65 per cent of the 234 cases) in which a study of an average of 3.7 slides per necropsy has shown a uniform histologic pattern in the primary tumor and in all secondary deposits. These cases are listed in the following four groups, each group containing cells of the indicated type exclusively.

Group A, Carcinomas Composed Entirely of Large Polygonal Cells (90 Cases). Large polygonal cells were found in closely packed masses 52 times, around poorly defined lumina 16 times, and in both arrangements 22 times.

Group B, Carcinomas Composed Entirely of Small Round Cells (8 Cases). The patients of group B tended to have an especially long clinical course but the metastases were always extensive, with involvement of the liver in 88 per cent of the cases.

Group C, Carcinomas Composed Entirely of Epidermoid Cells (38 Cases). Group C included 30 cases without pearls and 8 showing pearl formation. The relatively low level of metastasis in members of this group has been reported by others.[10,11] The liver was the site of metastatic carcinoma in only 13 per cent of these cases in contrast to 34 per cent for the series as a whole.

Group D, Carcinomas Composed Entirely of Columnar Cells (15 Cases). The number of women represented in the cases of group D was relatively high. These tumors tend to be more peripherally placed in the lungs than are tumors of other types.

In the remaining 83 cases (35 per cent of the entire series of 234) the growths contained more than one type of cell, either in the primary growth, in the metastatic foci, or in both locations, when studied in an average of 4.3 slides per necropsy. As there is no objective method of establishing which of the several cell types in these tumors is the significant one, thus enabling us to classify these cases under one of the four primary groups, these carcinomas with mixed morphologic features are placed in five additional groups.

Group A B, Carcinomas Composed of Both Large Polygonal and Small Cells (33 Cases). In group A B, small cells were found mixed

with large polygonal cells in closely packed masses 29 times, and with large polygonal cells arranged around poorly defined lumina, 4 times. In all of the necropsies listed in the subgroup of 29 cases, there were from a few to very many groups of cells in which large polygonal cells surrounded small round cells. This arrangement with peripheral larger cells and more centrally placed smaller ones was found in the bronchial walls, in the pulmonary alveoli, in the tracheobronchial nodes, and in the metastases in the liver, adrenal gland, kidney, and other organs. The larger peripheral cells were characterized by having more sharply defined edges and by appearing better preserved than those toward the center of the masses. The small cells in the center were indistinguishable from those already described under cell type B. Their cytoplasm was scanty. Their nuclei strongly resembled those of the large polygonal type except in those cases in which they had become pyknotic or showed further indications of necrosis. The point to be emphasized in this combined picture is that the larger cells at the periphery merged so closely with the centrally placed elements that it was impossible to say where one type ended and the other began. The histologic picture would seem to indicate that the more viable-appearing, larger cells at the periphery of the masses were undergoing a transformation to the smaller and often progressively necrotic cells toward their center.

A second interrelationship found among tumors with this composition may be described as follows. In 4 of the 29 cases, the primary tumor was composed of cells that were not clearly of large or small-celled type; that is to say, they contained a mixture of transitional forms. In the metastases in the liver, bone, or other organs, however, the carcinoma consisted exclusively of extremely large cells. This finding indicates to me that the larger cells are more likely to spread than the smaller ones.

Metastases were the rule in this group and were found in 94 per cent of the 33 cases. They were found in the liver in 52 per cent of the cases in this group.

A number of authors consider the large and small cells as closely related, if not identical. The two types, considered together, are described by Smetana, Iverson, and Swan[12] as "undifferentiated cell carcinoma," and by Willis[1] and Liebow[13] as "anaplastic." (Foot[2] used "anaplastic" to refer only to carcinomas composed of small cells.) D'Aunoy, Pearson, and Halpert[14] described "reserve cells," which appear from their illustrations to represent both of these types. If this concept had been used in classifying the cases from this medical center, the resulting group would have included 131 cases now listed under

groups A, B, and A B; 56 per cent of the entire series of 234 cases.

Group A C, Carcinomas Composed of Both Large Polygonal and Epidermoid Cells (18 Cases). The large cells entering into the combined picture of group A C were present in closely packed masses 15 times, around empty spaces 2 times, and in both arrangements once. Pearls were found only 3 times in the epidermoid cell component of this combined picture. The one most helpful finding in differentiating a mass of epidermoid cells from a mass of large polygonal cells was the presence of fine lines of eosinophilic tissue between the epidermoid cells. These were not found between the large polygonal cells. The similarity between epidermoid and large polygonal cells may be so marked as to make it seem unlikely that it is accidental, and to suggest that one type runs into the other. Many authors deny the possibility of this relationship. Most of the authors who postulate this transformation favor the concept that the transition proceeds from the epidermoid to the large polygonal cells.

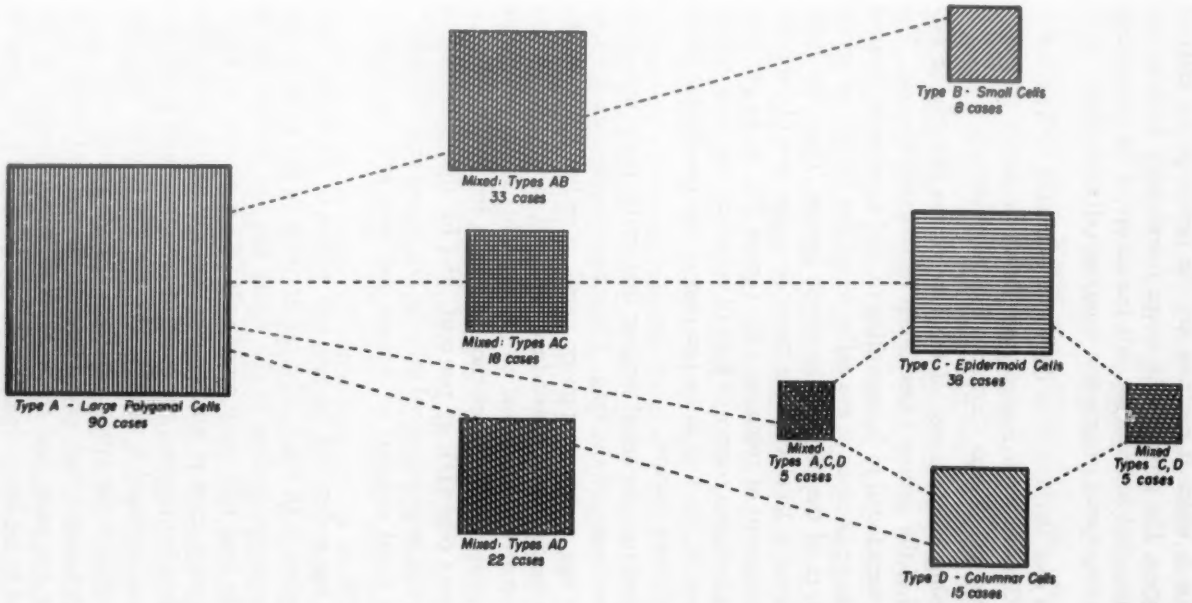
Group A D, Carcinomas Composed of Both Large Polygonal and Columnar Cells (22 Cases). The columnar cells of group A D were arranged in the typical manner previously described. The large polygonal cells were arranged exclusively in closely packed masses in only 2 cases. In 7 cases large polygonal cells were arranged exclusively around poorly defined lumina. In the other 13 cases, the large polygonal cells were found in both arrangements. In other words, when large polygonal cells were found in the same necropsies with columnar cells, the former usually were in a periluminal arrangement.

Group C D, Carcinomas Composed of Both Epidermoid and Columnar Cells (5 Cases). All of the members of group C D showed a histologic picture consisting of epidermoid cells without pearls and columnar cells. Phillips, Basinger, and Adams[15] found carcinoma of these two types together.

Group A C D, Carcinomas Composed of Three Types of Cells: Large Polygonal Cells, Epidermoid Cells, and Columnar Cells (5 Cases). The large polygonal cells of this mixed group were arranged both in closely packed masses and around poorly defined lumina 4 times, and exclusively in closely packed masses in the other case. The epidermoid cells showed no tendency to pearl formation.

Relationship Between the Various Groups

The nine groups are indicated diagrammatically in Text-figure 1, four containing cells of only one type and five containing cells of more than one type. Group A is placed at the left of the diagram not only



Text-figure 1. The squares represent the nine histologic groups into which the 234 cases of carcinoma of the lung were divided on the basis of the histologic constituents of each group. The number of necropsies in each group is indicated and the area of the corresponding square is proportional to this number. The four groups designated contained cells of the indicated type, exclusively: type A, large polygonal cells; type B, small round cells; type C, epidermoid cells; and type D, columnar cells. The rays extending from type A to types B, C, and D intercept the mixed groups A B, A C, and A D, respectively. The interrelationships of the two other groups C D and A C D are illustrated. In all cases the cross-hatching indicates the nature of the cells.

because it is so much the larger, but also because it is postulated that the cells of type A which it contains are the prototype for cells of all the other types. The groups with mixed morphologic features are placed in positions that indicate not only the nature of the constituent cells but also a hypothetic progression from one cell to another.

Similarities and Differences Between Members of Various Histologic Groups with Respect to Their Location in the Lung and Their Clinical Characteristics

The relationship of carcinoma of the lung to the primary or secondary bronchi of the various lobes should be commented upon. The larger bronchi were found in juxtaposition to, and apparently invaded by, carcinoma in 77 per cent of the entire series, but this figure varied with the members of the different histologic groups. Thus, the large bronchi were found to be close to the tumor in 53 per cent of the cases of carcinoma composed of columnar cells (group D); in 59 per cent of the cases of carcinoma composed both of columnar and large polygonal cells (group A D); and in 81 per cent of the carcinomas listed under the other seven groups.

The series confirms the usual finding that many more men than women have carcinoma of the lung. Pulmonary carcinomas are now the most numerous malignant tumors found in males, but only the ninth most frequently found tumor in women. Of the 234 cases, 195 (83 per cent) occurred in men and 39 (17 per cent) in women. There is, however, a relatively high incidence in women for the group composed of columnar cells (D), 47 per cent; and for that composed of columnar cells with large polygonal cells (A D), 32 per cent. Graham[16] reported about equal numbers of women and men with adenocarcinoma of the lung.

The average age at death of the men and women of the entire series considered together is 58 years. The age at death of the cases in the other histologic groups is close to this figure but the average age of carcinoma of the lung with small round cells (B) is greater, 64 years.

Except for a single case in which the patient survived 9 years after resection, the interval between the onset of symptoms and death was always short and averaged 6 months. The average interval was prolonged to 11 months in the group composed of small round cells.

The pulmonary cancer had spread beyond the regional nodes in 76 per cent of the 234 cases, but there was a definite difference in the extent of spread in the cases from the various groups. To list them in the order of the increasing number with metastases: Group D with

columnar cells showed widespread metastases in 67 per cent; group C with epidermoid cells, 68 per cent; group A with large polygonal cells, 69 per cent; group A C with large polygonal and epidermoid cells, 78 per cent; group A D with large polygonal and columnar cells, 91 per cent; group A B with large polygonal and small round cells, 94 per cent; and group B with small round cells, 100 per cent.

Discussion

Mallory,[17] Klotz,[18] Fischer,[9] Samson,[19] Frissell and Knox,[20] and others considered the bronchial epithelium the probable site of origin of carcinoma of the lung. Ewing[21] stated that carcinoma of the lung may arise from the bronchial epithelium, the bronchial mucous glands, or the alveolar epithelium, but that the specific features of the various types of tumor and their points of origin can be recognized only in the earlier stages of tumor development. The present study with its great preponderance of late cases does not give as definite an indication of the point of origin from the bronchial mucosa as has been demonstrated by Hutchison[8] in his series of earlier cases. However, the cells of the pulmonary carcinoma resemble those of normal bronchial epithelium much more closely than they do the cells of adult alveolar epithelium with their scanty cytoplasm and small nuclei.

Variations in the histologic pattern of carcinoma of the lung have been noted by many authors.[1,9,18] The explanations for these variations are numerous.

Some authors, including Foot[2] and Moersch and McDonald,[3] classified the tumors they described in as definite histologic groups as possible, but the latter authors indicated that their classification by predominant cellular type lacked complete objectivity when they wrote that "some large cell carcinomas are, in reality, highly undifferentiated forms of squamous carcinoma, while others represent highly undifferentiated forms of adenocarcinoma." Others, including Cahan, Butler, Watson, and Pool[22] and McGrath, Gall, and Kessler,[23] believed that cellular differences indicate presumptive multiple origins for the various types of carcinoma, each type preserving its characteristic structure throughout. While it is impossible to deny this thesis categorically, the frequent juxtaposition of only slightly varying tumor cells makes this explanation unsatisfactory. There was only one necropsy among the 234 from this hospital in which the evidence indicated a presumptive double origin of primary carcinoma of the lung.

Willis,[1] on the contrary, stated that "there is only one entity *carcinoma of the lung*, that individual tumours show various structural

combinations, and that great pleomorphism is common in one tumour."

Weller[24] postulated a transition from one cellular type of carcinoma of the lung to another by entdifferentiation.* He and Samson,[19] who expanded his series, illustrated their concept of the relationship of the various forms of pulmonary carcinoma by devising a Y-shaped classification of all of their tumors, in which each case was considered transitional between the one above and the one below it. The upper poles of the Y represented the more adult columnar and cornifying squamous tumors, respectively. The junction of the three arms represented the type of tumor with undifferentiated, relatively large cells. Tumors of an undifferentiated small cell type were placed at the base of the Y. The concept of these authors was that these tumors are all related in that each cell type represents neoplasia at a particular level of differentiation above the most primitive basic cell type. A somewhat similar explanation of the relationship of the cells is offered by Björk.[25] The theory of dedifferentiation has been invoked as an explanation for the more anaplastic cellular patterns found in cancer of many organs by von Hansemann[26] and Goodpasture.[27] It is not subscribed to by MacCallum[28] or Karsner.[29]

There is another possible explanation of the interrelation of diverse cellular patterns in carcinoma of the lung; namely, that the large polygonal cells are the primary type and that all others are variants of this type. The large polygonal cells are much the most numerous form in our material. A number of authors[17,30] believe that tumor cells of various organs possess a capacity of differentiation similar to that of the parent organ. This capacity apparently has not been exceeded in the tumors studied.

As previously indicated, many authors believe in the essential similarity of the large polygonal and small round tumor cells. The findings in the present series agree with the thesis that large polygonal cells may become transformed into small round forms. Certainly, the larger cells are found in just those areas where more advanced growth would be expected; namely, at the periphery of the masses composed of both types of cells and in the metastases. The smaller cells with their scant cytoplasm and pyknotic nuclei appear less capable of growth than the others. This low growth potential may explain the relatively long survival of patients with this type of carcinoma in spite of the wide dissemination of these tumor cells. Other examples of the progression

* Entdifferentiation was used instead of dedifferentiation to indicate lack of complete differentiation in the ascending scale from a common or basic cell of origin, rather than loss of differentiation already achieved.—*Editor.*

from larger to smaller cells are furnished by the maturation of lymphocytes and of the cells of malignant lymphomas.

I believe that there is also a development of the large polygonal cells (Fig. 2) into the more differentiated epidermoid cells as seen in Figures 6 and 7 rather than in the reverse direction. The relatively low incidence of metastasis found in carcinoma with epidermoid cells would seem to confirm the theoretic likelihood that the cells at the end of a process of maturation would be less likely to metastasize than their earlier progenitors.

Similarly, it is postulated that large polygonal cells, especially when arranged around poorly defined lumina (Figs. 3 and 4), develop into the pattern of columnar carcinoma as in Figure 9, rather than that the cells develop in the opposite direction. Even though the neoplasms with columnar cells (type D) are found in almost as many women as men and the same group has a more peripheral location in the lungs than other groups, these two factors do not justify a complete separation of these tumors from those of other types. This is particularly true since the mixed type, A D, is transitional not only in structure but also in incidence according to sex and in the peripheral location of the tumors between the group with large polygonal cells (A) and the group with columnar cells (D).

The findings as a whole can best be explained on the assumption that the large polygonal cells are the primary element in carcinoma of the lung and that these cells may persist as such or become modified to form cancers composed of small round cells, epidermoid cells, or columnar cells.

Summary and Conclusions

A histologic study has been made of 234 cases of carcinoma of the lungs and bronchi seen in the New York Hospital between 1925 and 1953.

The cells found in the carcinomas were of four types: large polygonal cells (type A), small round cells (type B), epidermoid cells (type C), and columnar cells (type D), the last mentioned including "adenocarcinoma" and "terminal bronchiolar carcinoma." Tumors composed exclusively of one or another of these four types of cells made up 65 per cent of the cases; growths containing mixtures of two or three types of cells were found in the remaining 35 per cent.

The large polygonal cells appeared to merge with small round cells, especially when they were found at the periphery of a mass of cells that contained the smaller cells near their center. Furthermore, the large polygonal cells sometimes were found alone in metastases from a pri-

mary tumor that contained both large polygonal and small round cells. Hence the possibility was considered that the large polygonal cells might often be transformed into small round cells. In a number of instances, moreover, the large polygonal cells were found in close apposition to epidermoid or columnar cells, or both, the findings suggesting that the large polygonal cells may also give rise to cells of epidermoid or columnar type.

In view of the mixtures of cells found in a large proportion of the growths and the apparent differentiation of the large polygonal cells into cells of other types, it appears that carcinoma of the lung, though obviously presenting various histologic pictures, should be regarded as a single entity.

The photomicrographs were taken by Mr. Julius Mesiar.

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[*Illustrations follow*]

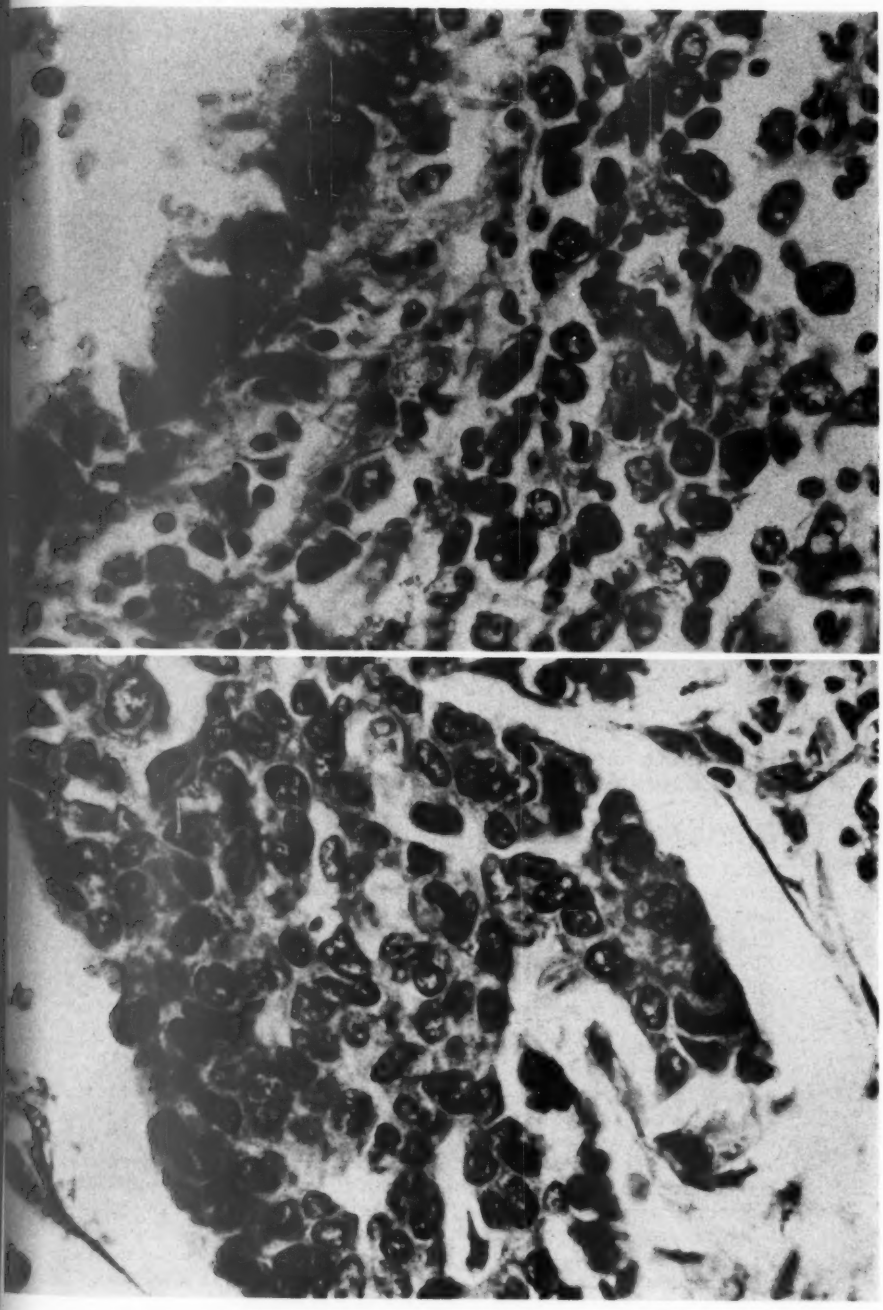
LEGENDS FOR FIGURES

FIG. 1. A man, 75 years old, had a cough for 3 months before he died. A few acid-fast bacilli were found on an examination of concentrated sputum. At necropsy, a mass of carcinoma, 6 cm. across, was found to surround the main bronchus to the apical portion of the right upper lobe. Carcinoma had spread to the lungs and the regional nodes and there was right hydrothorax. There was inactive tuberculosis at the left apex.

The intact bronchial mucosa is shown in Figure 1. Underlying it there are masses of large polygonal cancer cells that extend throughout the tissue. The general resemblance in size and appearance between the cells of the normal bronchial epithelium and the tumor cells is apparent. The smaller cells are lymphocytes. Large polygonal cells in compact masses also were found in the lung and in a lymph node. × 600.

FIG. 2. A woman, 47 years of age, had lumbar pains and tingling sensations in her right ankle for 3 months before she died. At necropsy, there was a mass of carcinoma that occupied most of the upper lobe of the left lung and apparently arose from a bronchus of medium size. There were extensive metastases in the liver and also metastases in an adrenal gland, a kidney, and several vertebrae. Death was due to pulmonary embolism. Microscopically, the lung contained large polygonal cells arranged both in masses and around poorly defined lumina. The cells in the latter arrangement merged with characteristically appearing columnar cells. A similar picture was present in the nodes. In the liver and kidney there were large polygonal cells in both arrangements but no columnar cells. In the adrenal glands there were large polygonal cells arranged around poorly defined lumina and columnar cells.

Figure 2 shows a compact mass of large polygonal cells within a pulmonary alveolus. × 600.



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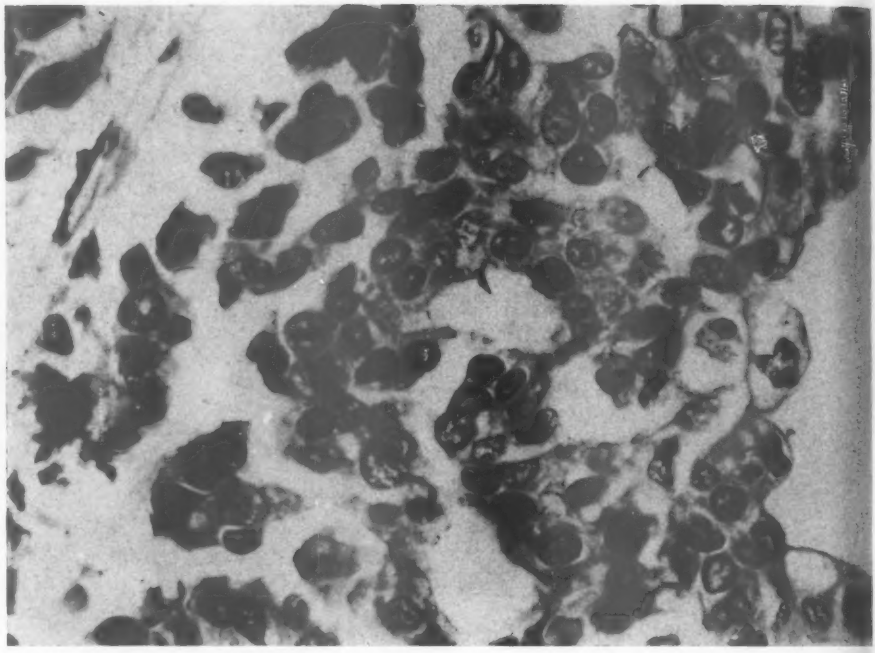

FIG. 3. From the same case as that from which Figure 2 was taken. Figure 3 shows an intra-alveolar area where the large polygonal cells are arranged around poorly defined and irregular lumina. × 600.

FIG. 4. A man of 51 years had intense pain in his left shoulder and chest for 8 months before he died. Aspiration material from the apex of the left lung in two outside hospitals showed carcinoma. Irradiation gave no relief. A cordotomy under endotracheal anesthesia was performed for pain but the patient died during the operation. At necropsy, a tumor mass, 5 cm. across, in the upper lobe of the left lung surrounded a small bronchus. There were metastases in the pleura, nodes, ribs, and spinal dura. Sections from the pleura, lymph nodes, ribs, and spinal dura showed the neoplasm illustrated in Figure 4, which is a photomicrograph from a metastasis in a lymph node. Large polygonal cells are arranged around irregular lumina. × 600.

FIG. 5. Twelve months before a man of 77 years died, paralysis of the left vocal cord developed. A persistent cough began 3 months prior to death. Irradiation with 4,800 r. caused a decrease in the size of a mass in the mediastinum. At necropsy, there was a carcinoma of the main bronchus to the upper lobe of the left lung 2 cm. from its origin, with stenosis of this bronchus. Carcinoma had extended to both lungs and to the tracheobronchial nodes. These involved nodes surrounded the left recurrent laryngeal nerve. There were many tumor nodules in the liver, in the peritoneum, in the 12th thoracic vertebra which was the site of a neoplastic fracture, and in the brain. All of the metastases were exclusively composed of small round cells. × 600.

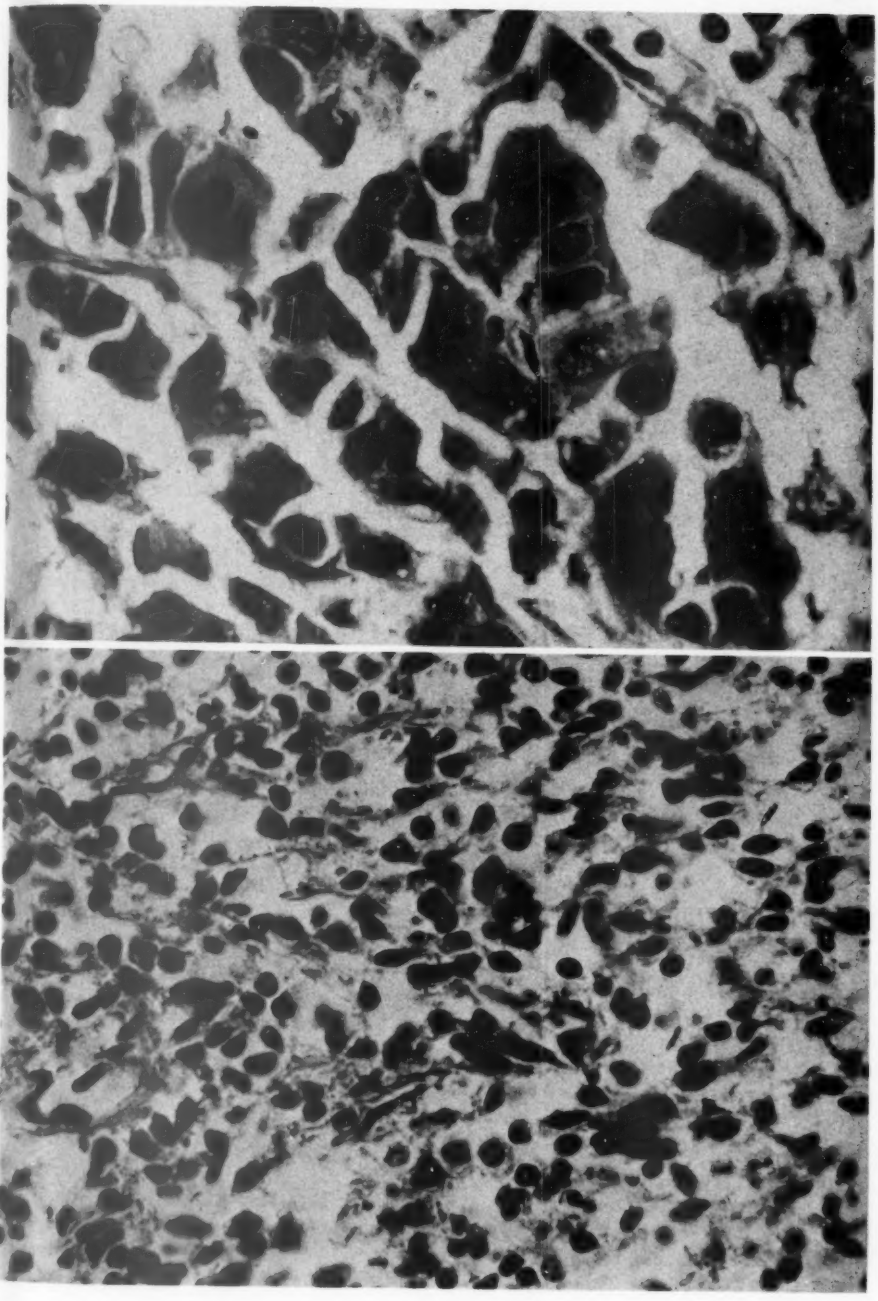

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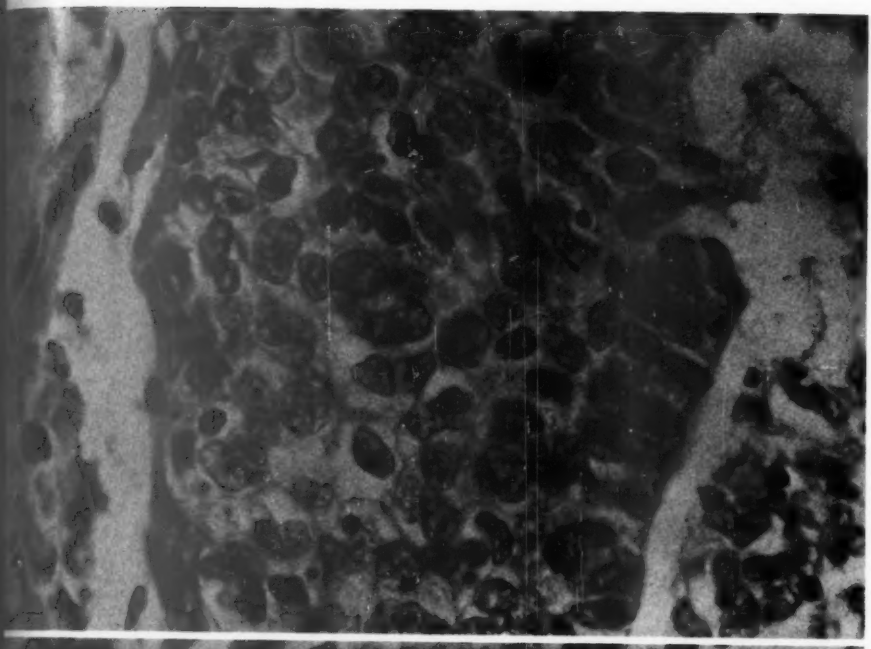
FIG. 6. A man of 53 years had a history of bronchitis and emphysema of 12 years' duration. He had pain in his chest for 18 months and biopsy of a bronchus showed carcinoma. There was no resection. Death was due to confluent bronchopneumonia. At necropsy, there was a large carcinoma in the lower lobe of the left lung, but there were no metastases. Microscopically, there were large polygonal cells both in masses and around poorly defined lumina in addition to the epidermoid carcinoma shown in Figure 6.

The field shows epidermoid carcinoma without pearls, of a relatively uniform pattern. The cells are closely packed and a definite line of demarcation is apparent between each of the adjacent cells. The material forming this line of demarcation is very pale red in hematoxylin and eosin preparations. Intercellular bridges cannot be identified. × 600.

FIG. 7. A man, 57 years old, had pain in his sacral region for 3 months and a metastatic tumor in the left supraclavicular fossa with Horner's syndrome for half that time. The sacral and cervical regions were irradiated. At necropsy, there was a carcinoma of the main stem bronchus to the lower lobe of the left lung. Death was due to bronchopneumonia and pulmonary infarction. There were a few small tubercles in the lung. The carcinomatous metastases in the liver, adrenal glands, kidneys, and sacrum showed the histologic picture illustrated.

Figure 7 illustrates a much more irregular type of pavement epithelium than was shown in Figure 6. Fine lines surround the cells and separate them from one another. (They stain pale red with hematoxylin and eosin.) There were neither intercellular bridges nor pearls. The tumor shown was found adjacent to a large bronchial cartilage. × 600.



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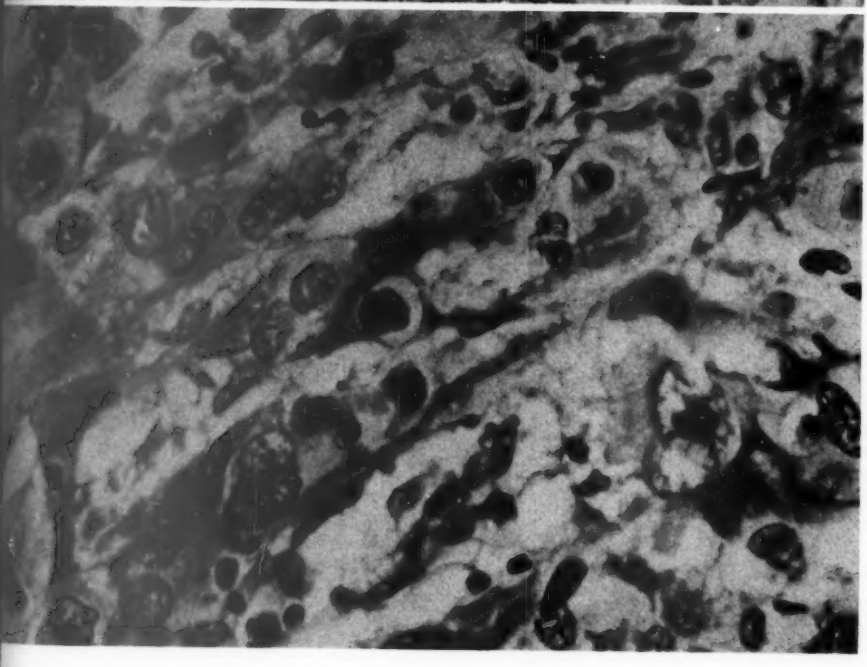

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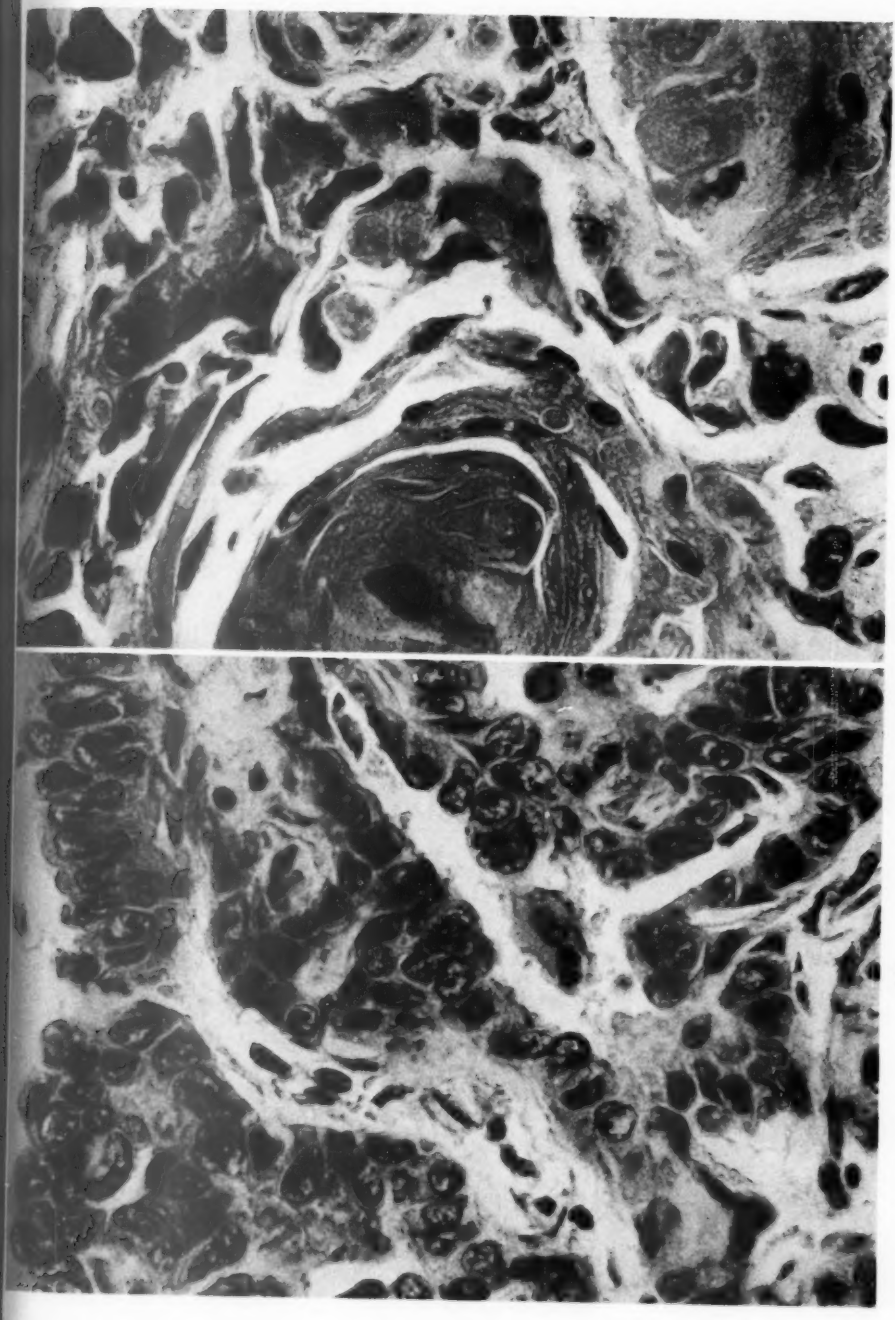
FIG. 8. A man of 39 years had an attack of pneumonia 6 months before he died and suffered from fatigue and thoracic pain for 2 months. At necropsy, there was a tumor around the bronchus to the lowest lobe of the right lung, with extensive necrosis. The esophagus was invaded by tumor and had perforated. There was fibrinous pleurisy, pneumonia, and pericarditis. One of the atria was invaded by tumor. Tuberculosis was present in the lungs and in the left adrenal gland. Microscopically, the tumors in the bronchus, lung, and esophagus all showed histologic pictures similar to that seen in the photomicrograph. An intrapulmonary focus is composed of a combination of epidermoid carcinoma with pearls and an adjacent mass of large polygonal cells in a compact form. × 600.

FIG. 9. A woman, 47 years old, had pain in the lumbar region for 8 months but she never had pain in the anterior part of the thorax nor cough. Laminectomy and cordotomy were performed in the region of the 12th thoracic vertebra for pain, and the tumor there was irradiated at the time of operation. She died 2 months later with bronchopneumonia and cystitis. At necropsy, there was a tumor mass, traversed by two bronchi, in the anterior inferior part of the upper lobe of the left lung. There were metastases in the left pleura with hemothorax, in the right lung, nodes, liver, spleen, one of the vertebrae, and in the meninges of the brain and cord. All metastases showed the arrangement of cells indicated in Figure 9, which was taken from an intrapulmonary mass composed of nests of carcinoma cells surrounded by thin strands of fibrous tissue. The regular arrangement of the columnar cells and the sharp definition of the lumina are well shown. It is of interest that an entirely similar structure was found at one point directly under the mucosa of a bronchus large enough to contain cartilage in its wall. × 600.



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STRUCTURAL FEATURES OF GOITERS IN SPORADIC CRETINS*

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Cretinism is due to a congenital deficiency of thyroid hormone. It is endemic or sporadic. Endemic cretinism is related to iodine deficiency and is associated with a nodular colloid goiter with more or less degeneration, and with or without reactive hyperplasia. Sporadic cretinism is unrelated to iodine deficiency. It usually is due to aplasia or atrophy of the thyroid gland, sporadic cretins with goiter being relatively uncommon.

The reported cases of goitrous sporadic cretins, in whom functional and histologic studies have been carried out, display paradoxic features. Therapy usually has been too little, too late, and often intermittent. Goiter may be first noticed as early as 5 years of age; it tends to enlarge progressively whether therapy is continued or not, and it is not influenced by the administration of iodine. Iodine-131 uptake usually is in the normal or hyperthyroid range and, while the iodide appears to be bound in some of these goiters, others release a large part of it promptly after the administration of potassium thiocyanate.[1-6] Blood cholesterol usually is elevated and the basal metabolism is low, though exceptions are reported. Previous replacement therapy probably accounts for some of these discrepancies (Table I).

The structure of the thyroid gland in the goitrous sporadic cretin is so striking that, having examined the surgical specimen in the first of the two cases herein reported and having compared it with the report of Stanbury and Hedge,[3] a specific diagnosis was possible when the sections from case 2 (B. L.) were examined.

REPORT OF CASES

Case 1

R. M. was a white male who had been born in Chicago in 1925. The diagnosis of cretinism was made at 7 years of age, and treatment with 90 mg. of desiccated thyroid daily was instituted with a good growth response. At age 16, the dose of thyroid was reduced to 60 mg. Goiter was first noted at 19 years of age, increasing progressively in size, so that the patient began to experience difficulty in breathing the following year. Lugol's solution, 2 cc. daily, in addition to the 60 mg. of desiccated thyroid, did not modify the progressive enlargement of the thyroid gland nor stay the increasing respiratory difficulty, which necessitated hospitalization.

* Supported by funds furnished by the Nelson M. Percy Medical Research Foundation.
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TABLE I

Data on Nineteen Cases of Sporadic Cretinism with Reference to Replacement Therapy

Author	Case	Sex	Age*	Age when symptoms were first noted	Age when replacement therapy started	Age when goiter was first noted	Size of goiter	Cholesterol	Basal metabolic rate	I-131 uptake	KSCN effect	Protein bound I	Miscellaneous
			yrs.					*mg./100 ml.*		%			
Hamilton *et al.*[1]	P.H.		4½	Birth			50 gm.†	328		2.2‖ 52.0¶			Thyroxin and diiodothyronin extracted from thyroid gland
	A.D.	M	19	1 or 2 yrs.			Slightly enlarged†	550	−41	0.55‖ 30.0¶			
Lerman *et al.*[3]	R.W.	M	19	10 yrs.	½ gr. at 10 yrs., 1 gr. later; ended at 17 yrs.	12 yrs.; increased after 17 yrs.	15 x 8 cm.†	169	−28	87			I content of thyroid, 0.07%
	C.W.	M	12	5 yrs.	½ gr. at 5 yrs., 1 gr. later; ended at 11 yrs.	5 yrs.; increased after 11 yrs.	Large, soft, nodular	183	−20 (at 11 yrs. after treatment, +9)				
	H.E.	M	13		Thyroid and iodine many years		240 gm.‡						
	D.B.	F	21				160 gm.‡						
Stanbury and Hedge[5]	M.E.	F	16	6 mos.	2 yrs. sporadically	7 yrs., progressive	97 gm.‡	212		Plateau in 2 hrs.#	Down 75% in 2 hrs.	1.02 μgm.	Thyroid inorganic I, 29.3 μgm. %; protein-bound I, 10.4 μgm.%
	C.E.	M	23	Birth	3 mos. sporadically	13 yrs., progressive	497 gm.‡	272		Plateau in 4½ hrs.#	Down 70% in 2 hrs.		Thyroid inorganic I, 173 μgm. %; protein-bound I, 15 μgm. %
	R.E.	F	13	Early	1 gr. sporadically		3 times normal†			Rapid#	Down 90% in 1 hr.		

Hubble[4]	G.W.K.	F	17	Before 7 yrs.	8 yrs., ended at 15 yrs.	15 yrs.		316 125 (post-operative)	−23				Thyroxin in thyroid gland and plasma
	G.K.	M	13	4 yrs.	½ to 1½ gr. at 4 yrs., 3 gr. at 13 yrs.			266 144 (after treatment)		60− in 12 hrs.	Small	70% plasma I	
	P.K.	F	8½	1 yr.	½ gr. at 1 yr.§	8½ yrs.		284 125 (after treatment)					
	J.K.	F	6½	1 yr.	½ gr. at 1 yr., 1½ gr. at 6 yrs.			259 140 (after treatment)					
Wilkins *et al.*[7]	D.G.	F		3 mos.	After 7 mos.		Normal			Normal**	None		
	R.G.	M	3	Birth	½ gr. at 11 mos., 1½ gr. at 3 yrs.		Normal	420 to 460		Normal**	None		
	A.B.	F	6¾	Birth	6¾ yrs.	6 yrs.	1½ times normal†	420		Normal**	None		
	D.W.	M		Birth	¼ gr. at 5 mos., ½ gr. at 14 mos.	8½ yrs.	40 gm.‡	320		Low			Diagnosis: carcinoma without recurrence or metastasis
Milles	R.M.	M	20	7 yrs.	1½ gr. at 7 yrs., 1 gr. at 16 yrs. plus I at 18 yrs.	18 yrs., progressive	150 gm.‡	117 after treatment					
	B.L.	F	19	1 yr.	1 yr.	13 yrs., progressive	32 gm.‡	180 at 18 yrs., 290 at 19 yrs.	−15 at 16 yrs., −5 at 19 yrs.				

* At time of report. † Clinical estimate.
‡ Weight of surgical specimen. § Tolerates no more than 1 gr.
|| Without carrier. * With carrier.
‡ The type of curve was similar to that seen in patients with hyperthyroidism. The uptake was rapid and complete by the fourth hour. In the next 24 hours there was a rapid loss of about three fifths of the accumulated I-131 and during the succeeding 4 days a gradual loss of small amounts.
** I-131 intravenously.

The patient was admitted to Augustana Hospital on the service of Dr. Nelson M. Percy in 1945. He was described as mentally retarded, clumsy, small of stature, and with a gibberish speech. He weighed 95 lb., his tongue was somewhat increased in thickness, the thyroid gland was asymmetrically enlarged, and the trachea deviated to the right. Wheezing râles were heard in both lung fields. Blood pressure was 85/55 mm. of Hg; pulse, 76; red blood cell count, 4.3 millions; hemoglobin, 82 per cent. A coarsely bosselated thyroid gland, weighing 150 gm., was excised. One week postoperative the blood cholesterol was 117 mg. per 100 cc. He was discharged on 0.3 gm. of proloid daily (equivalent to 0.3 gm. of desiccated thyroid) after an uneventful postoperative course. He had improved in mentality and had increased somewhat in size 8 years later (Figs. 1 to 4).

Case 2

B. L. was a white female secretary who had been born in Chicago in 1934. The diagnosis of cretinism was made at the age of 1 year and treatment with enteric coated tablets of desiccated thyroid, the exact dose of which is not known, was instituted. At age 13, enlargement of the thyroid gland was first noted and it was said to have been progressive. One year later, the patient developed a speech defect characterized by a nasal twang which, though not improved by attendance at a speech clinic, did not prevent her from finishing high school. At 16 years of age, she was examined at the Mayo Clinic, where a diagnosis of cretinism with goiter, partially controlled by therapy, was made. At that time her weight was 113½ lb. and blood pressure, 92/70 mm. of Hg. The thyroid gland was the site of soft nodules. The right lobe was estimated to be 3 by 3 by 2 cm.; the left, 1 by 2 by 1 cm. The basal metabolic rate was minus 15 and the blood cholesterol was 180 mg. per 100 cc. Bone age, estimated roentgenographically, was compatible with the stated age. Psychologically, she was classified as dull normal.

At 19 years of age, she was admitted to Augustana Hospital on the service of Dr. Nelson M. Percy. The facies was cretinoid and speech was somewhat guttural. Menstrual history was normal. Blood pressure was 95/55 mm. of Hg; pulse, 88. The thyroid gland was enlarged, more markedly to the right, and was nodular. Excision was made of 32 gm. of coarsely bosselated thyroid gland. The cut surface was made up of nodules measuring as much as 2.5 cm., with a small amount of intervening, delicately lobulated tissue.

On the sixth postoperative day, the basal metabolic rate was minus 5; weight, 111 lb.; height, 61 inches; pulse, 76; blood cholesterol, 290 mg. per 100 cc. with 75 per cent esters. She was discharged on 0.12 gm. of desiccated thyroid daily, after an uneventful convalescence.

Pathologic Findings

The gross and microscopic anatomy of the thyroid glands from these two cretins and the findings of other authors, though differing somewhat in minor details, are sufficiently constant to permit a composite description. They vary in size from clinically normal (patients D. G. and R. G. reported by Wilkins *et al.*[7]) to 497 gm. (patient C. E. reported by Stanbury and Hedge[8]), and are nodular and asymmetric, though the latter features may not be evident clinically if the enlargement is not pronounced. The cut surface is subdivided into delicately encapsulated nodules of varying size, whose medullary texture may be partially obscured by hemorrhage in areas. The internodular tissue is

scant, delicately lobular, and medullary to myxomatous, totally lacking the "beefy" texture of the normal thyroid gland. The surface is ivory colored shaded with tan, pink, and dark red, the red color resulting from intranodular hemorrhage.

Taken as a whole, the histologic pattern (Figs. 1 to 11) is diagnostic. Each nodule, enclosed in a delicate capsule, has a uniform make-up which, in one instance, is that of a diffuse, untreated hyperplastic goiter (A)*; in another, that of a fetal adenoma (B); in another, that of an embryonal adenoma (C); in another, an essentially normal thyroid gland (D); and in others, of pleomorphic cells, whose nuclei are, in many instances, bizarre and hyperchromatic. These cells are arranged in nests and cords, and in follicles containing thin, vacuolated colloid (E). Other nodules consist of flat cells arranged in poorly formed follicles of varying sizes, strikingly free of colloid (F). Centers of large, pale cells in small, colloid-free follicles, or in nests, are seen within some nodules, usually of the fetal type. The internodular tissue varies in amount and is unique (H). It consists of sharply defined, angular lobules of moderate size, compressed by the nodules, separated by a scant, delicate stroma, and composed of pleomorphic cells whose cytoplasm is light pink (hematoxylin and eosin stain), like ground glass or foamy in consistency, with oval vesicular to cinder-like pyknotic, occasionally giant nuclei. The organization of these cells into small groups or follicles devoid of colloid is poorly defined, and rarely includes a normal follicle. The stroma is scanty and only moderately vascular. Lymphoid tissue is notably scant or entirely absent.

A diagnosis of carcinoma without metastases or recurrence was made in one reported case (patient 4).[7] In my opinion this diagnosis was based on one of the bizarre, though non-neoplastic, areas. Indeed, if the definition of a neoplasm as an autonomous growth of tissue is accepted, even the term adenoma probably is not appropriate in describing these goiters.

Discussion

The majority of sporadic cretins are athyroid. Deficiency of iodide accounts for endemic cretinism and may be the determinant in some sporadic cases (Wilkin's[7] patient 4). The majority of the cases studied by the tracer technique displayed a normal uptake (A. D.[1] and D. G., R. G., and A. B.[7]) or an uptake in the hyperthyroid range (P. H.[1] and M. E., C. E., and R. E.[8]). The rapid release of the trapped iodide following the oral administration of thiocyanate in the cases reported by Stanbury and Hedge[3] is due to the failure of these thyroid glands

* The capital letters are repeated in the legends of the photomicrographs.

to bind the iodide. Hamilton *et al.*[1] demonstrated thyroxin and diiodothyronin in the thyroid glands of two sporadic cretins, and Stanbury and Hedge[3] found significant amounts of protein-bound iodine in the thyroid glands of two patients, one of whom had a significant amount of protein-bound iodine in the serum, a finding also reported by Hubble.[4] The presence of the hormone in the thyroid gland and not in the serum would suggest a failure on the part of the thyroid gland to release it, while its presence in the serum suggests a failure on the part of the tissues to utilize it due either to a tissue fault or to the formation of an abnormal hormone. The possibility that these findings are related to prior therapy cannot be overlooked, but is unlikely in view of the fact that nodules of thyroid tissue histologically indicative of active function are a constant finding. Thus we are forced to assume that failure to produce thyroid hormone is not complete but is quantitatively or qualitatively inadequate, in terms of either production, release, or utilization. The fact that the bulk of the cretinous goiter is histologically abnormal suggests a low functional capacity on the part of the gland.

The etiology of sporadic cretinism is unknown. The iodine-deficient goiter is of the colloid type modified by degeneration, while the goitrogens, including cobalt[8] and nutritional deficiency,[9] result in a diffuse, rather than nodular, enlargement. It is noteworthy, moreover, that every attempt to implicate the ingestion of a known goitrogen as a cause of thyroid enlargement in cretins has failed.

Gardner, in a discussion of a paper by Wilkins[10] on *The Development of Goiters in Cretins without Iodine Deficiency,* stated that this group probably represents a "genetic recessive trait." Though many of the reported cases do occur in siblings,[2-5] a common environmental factor is equally probable.

Replacement therapy, usually inadequate and often intermittent, is common to the reported cases and either alone or in conjunction with some other factor appears to determine these goiters. Certain nodular goiters in adults, to which the term adenomatous is applied, bear a sufficient resemblance to the group under discussion to suggest a common, though at present unknown, etiology, modified in the sporadic cretin by the metabolic needs of infancy and childhood.

Summary

The microscopic structure of the goiter of the sporadic cretin is diagnostic of that state on the basis of the kaleidoscopic pattern of hyperplastic, fetal, embryonal, and bizarre nodules, and internodular,

sharply defined, angular lobules of pleomorphic cells in a comparatively scant stroma notably lacking in lymphoid tissue.

The high uptake of iodide and its rapid release following the administration of thiocyanate is indicative of inability on the part of these goiters to bind iodide, a fact which is difficult to correlate with the presence of a significant amount of histologically functional nodules unless it is assumed that a defect in the mechanism for the release of thyroid hormone also exists.

The goiter of the sporadic cretin can be compared histologically with that of the so-called adenomatous goiter of the adult, suggesting the possibility of a common etiology, whose effect has been modified by the exigencies of infancy and childhood.

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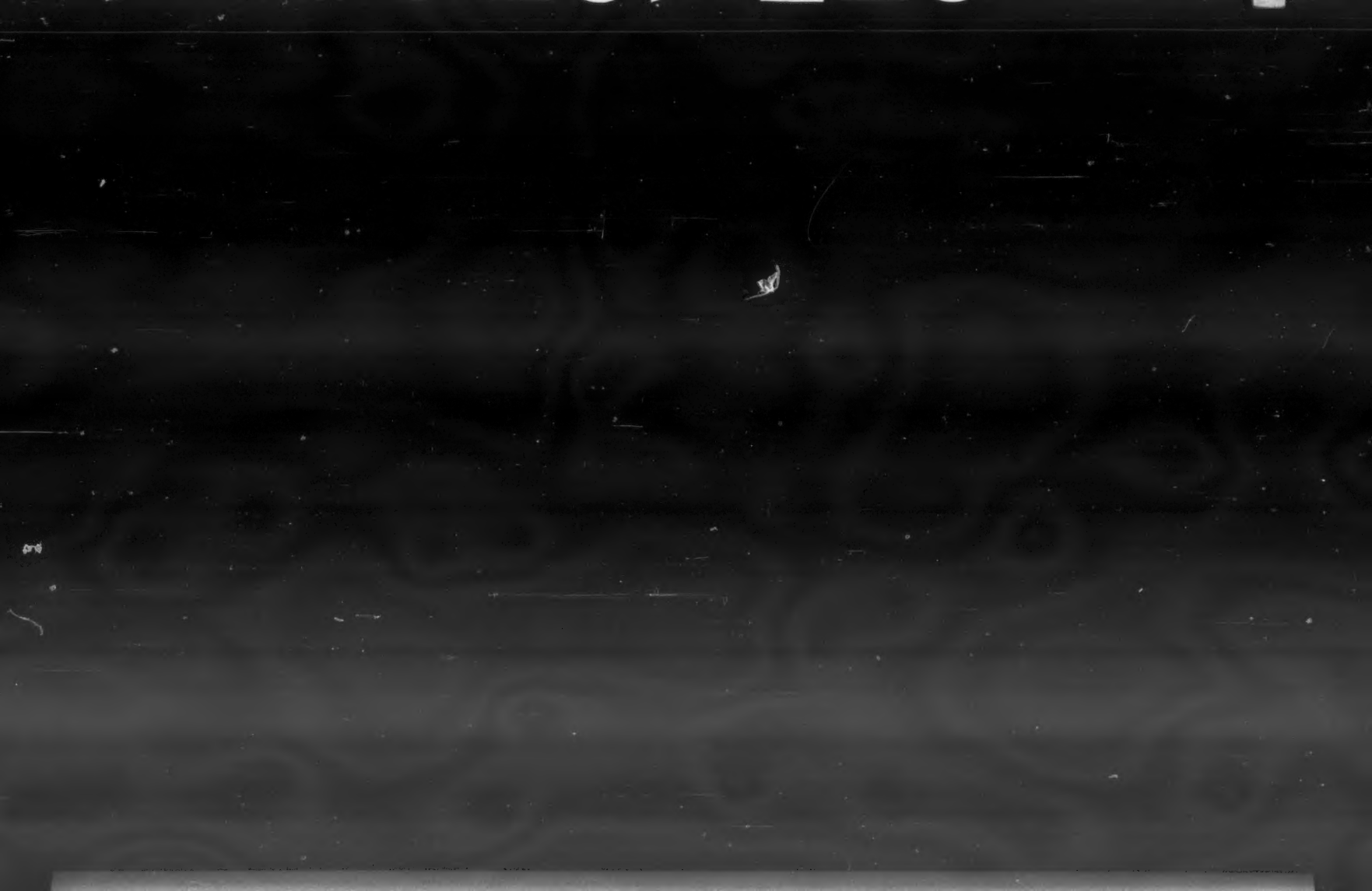
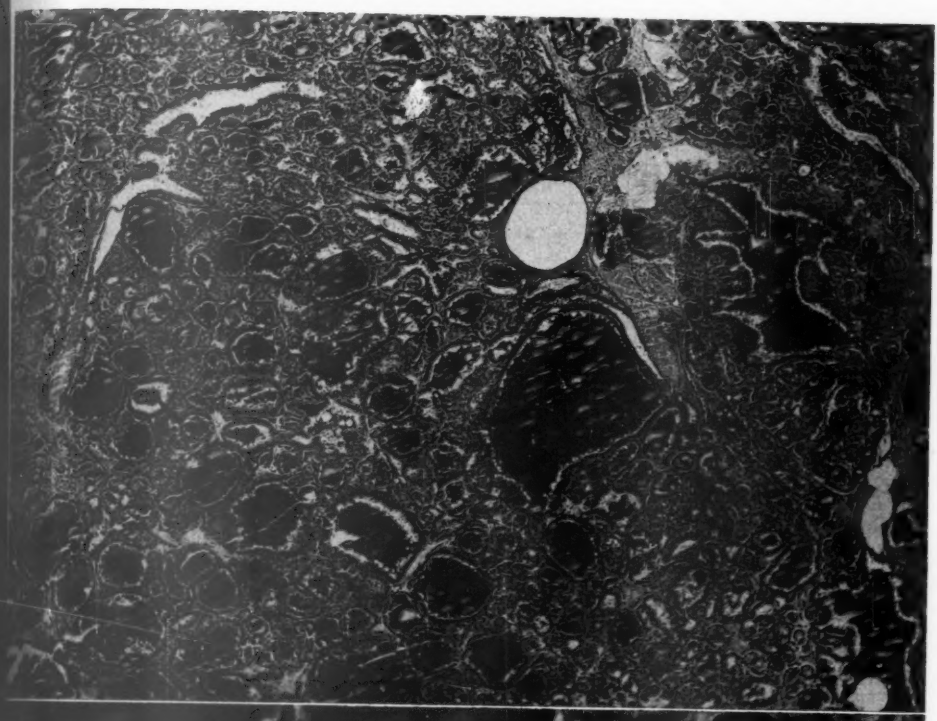
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Case 1. Hyperplastic nodule, type A, as referred to in the text. × 65.

FIG. 2. Case 1. Hyperplastic nodule, type A. × 255.





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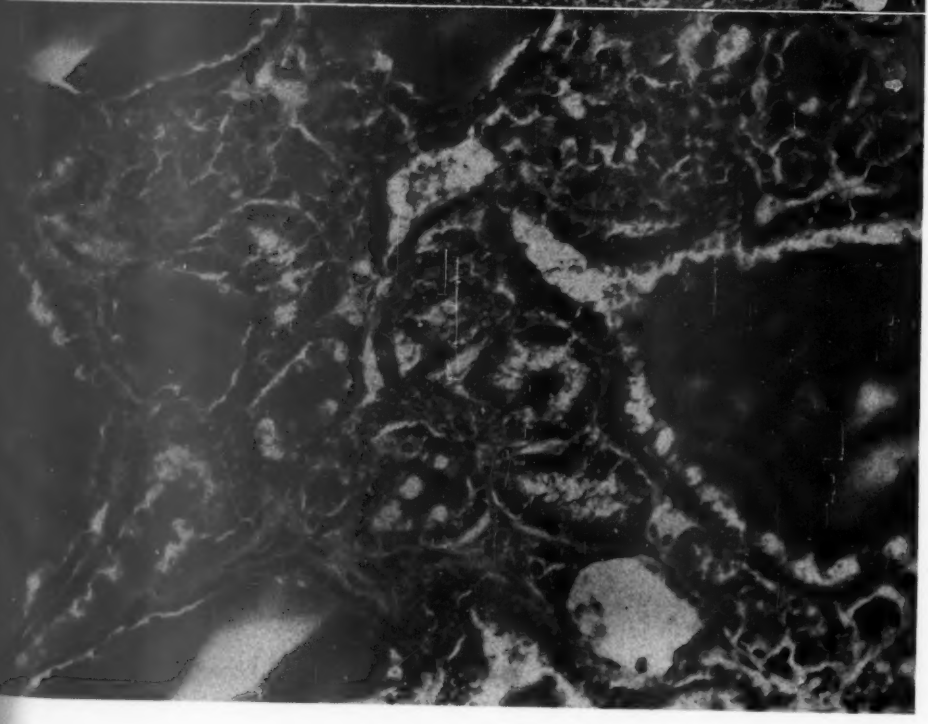

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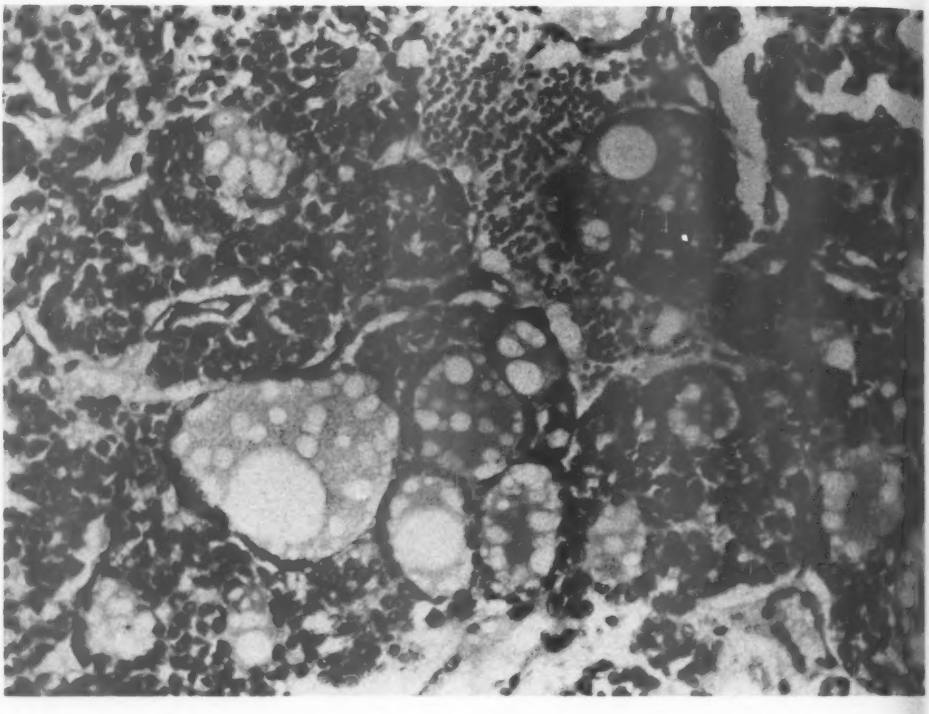
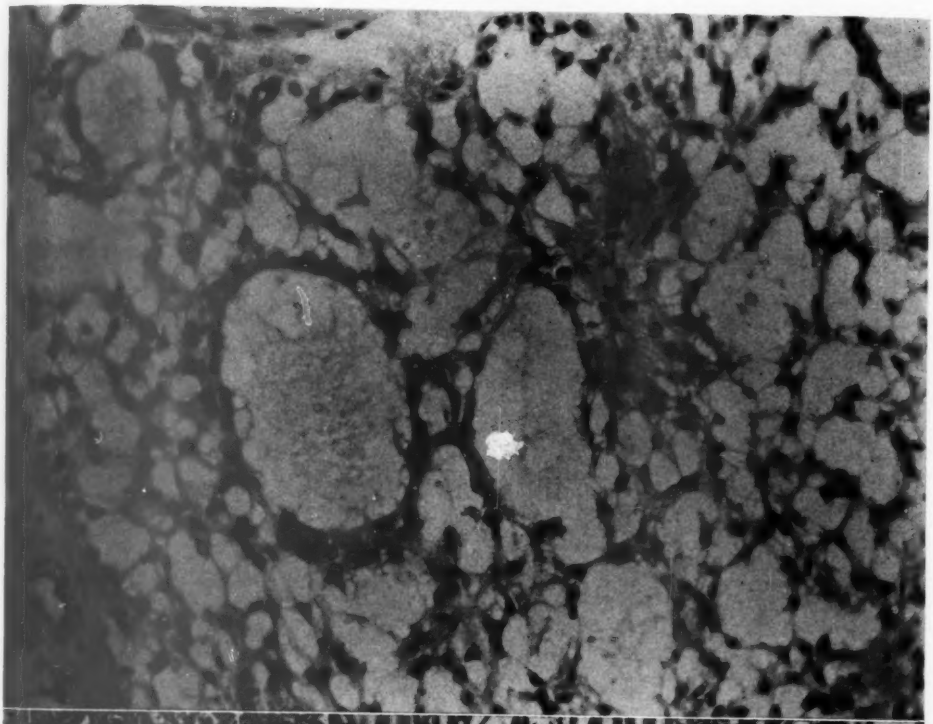


FIG. 3. Case 1. Pleomorphic cells in nests, cords, sheets, and follicles containing thin, vacuolated colloid or devoid of colloid. Type E, as referred to in the text. × 255.

FIG. 4. Case 1. Flat cells lining poorly formed follicles of varying size, practically devoid of colloid. Type F. × 255.

FIG. 5. Case 2. Hyperplastic nodule at the left, type A; "fetal" nodule at the right, type B; internodular, sharply defined, angular, compressed lobules of poorly organized, pleomorphic cells, type H, in central portion of field. × 65.



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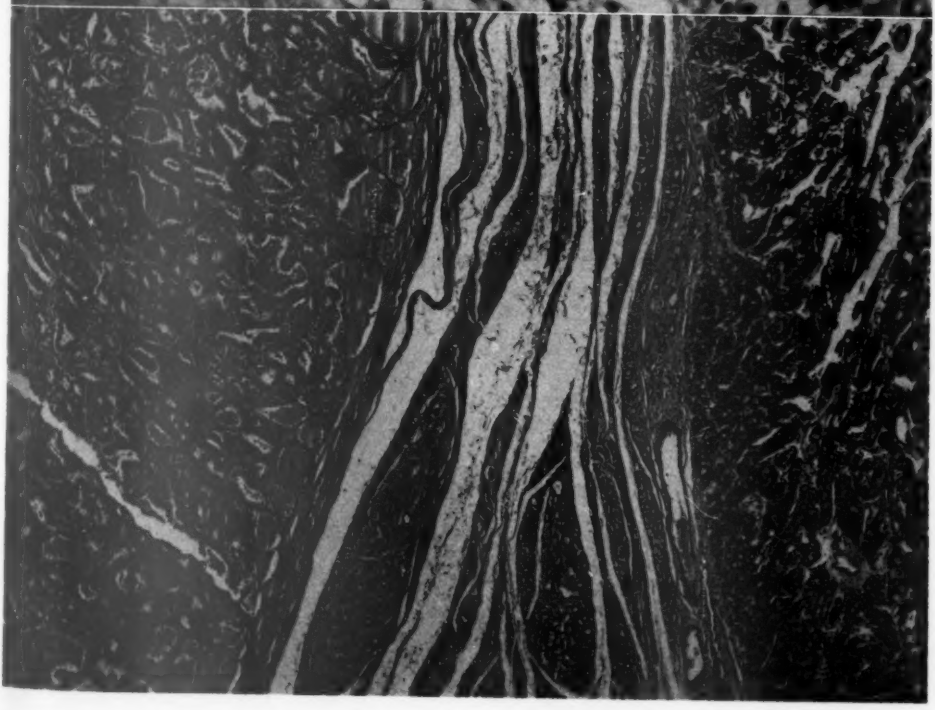

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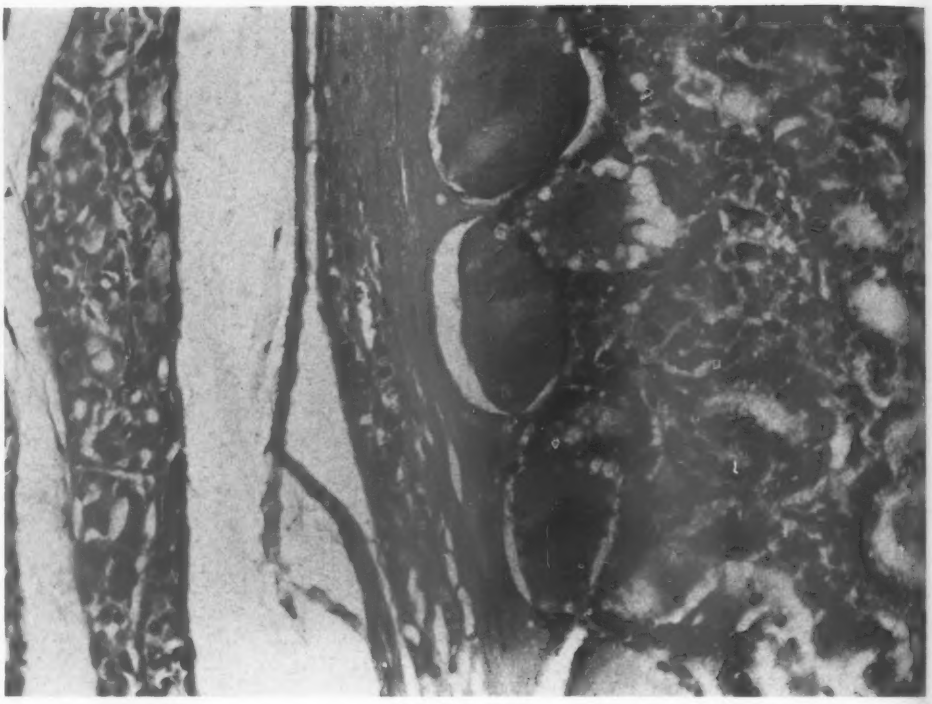
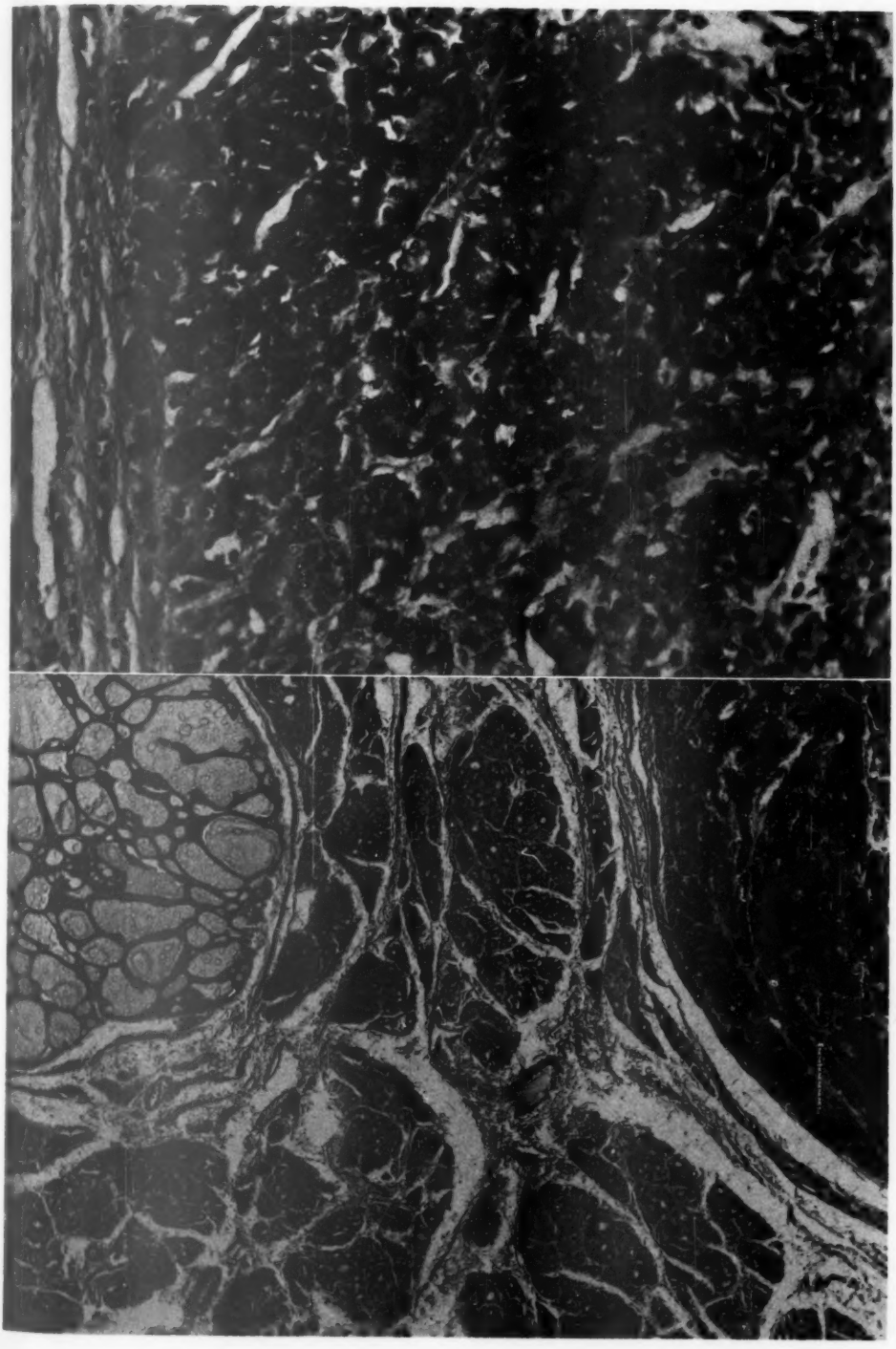


FIG. 6. Case 2. Hyperplastic nodule, type A, in right half of field; internodular, sharply defined, angular, compressed lobules of poorly organized, pleomorphic cells, type H, at extreme left. × 255.

FIG. 7. Case 2. "Fetal" nodule, type B. × 255.

FIG. 8. Case 2. Nodule of normal thyroid gland, type D, at upper left; "embryonal" nodule, type C, at upper right; multiple, internodular, sharply defined, angular, compressed lobules of poorly organized, pleomorphic cells, type H, in central and lower portions of the field. × 65.



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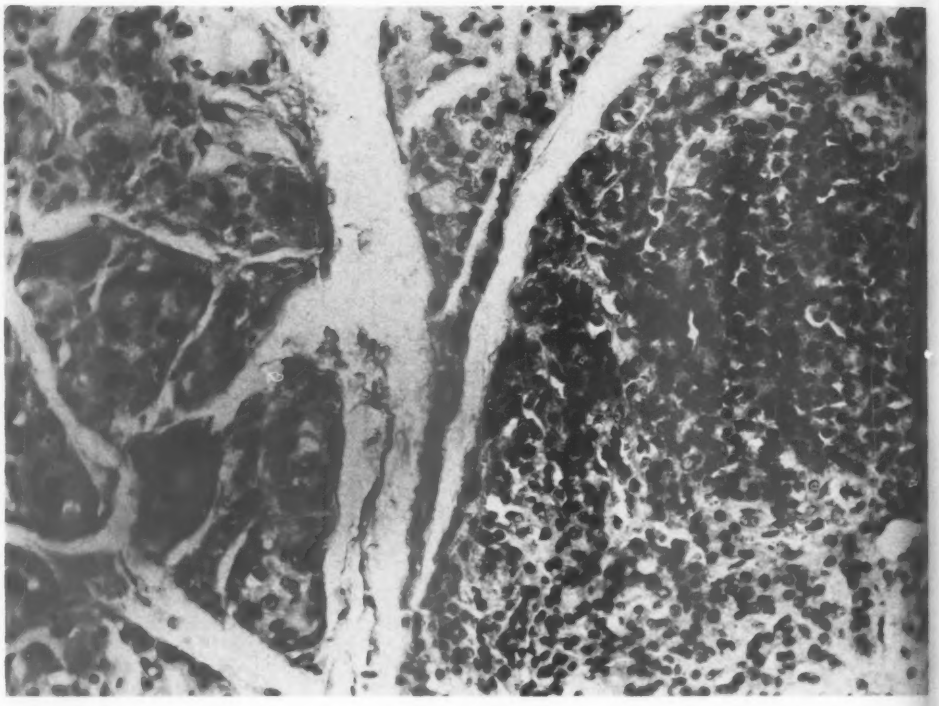
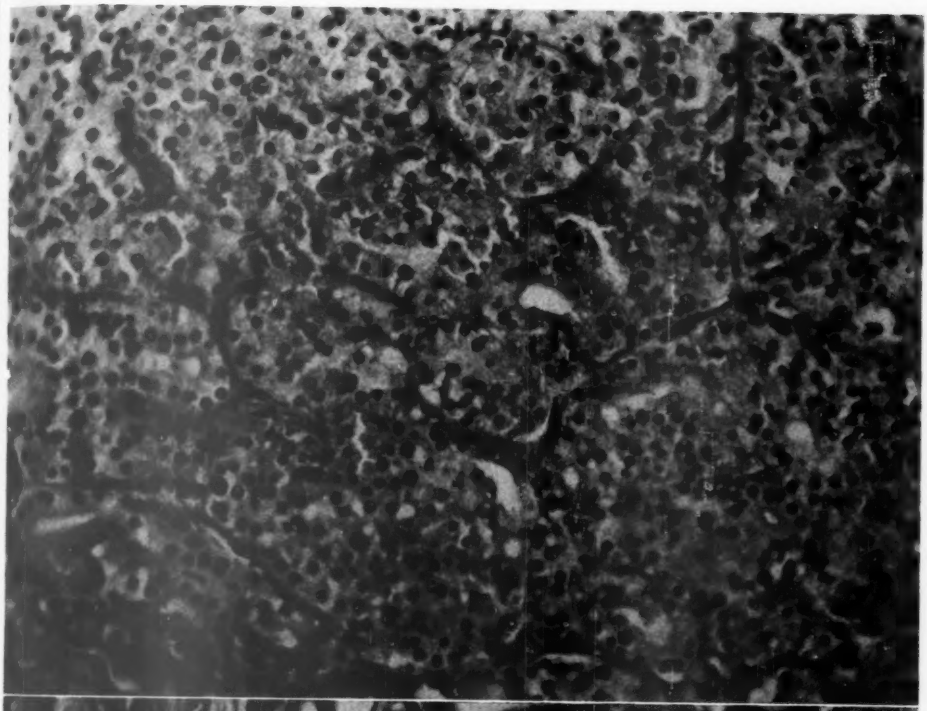


FIG. 9. Case 2. "Embryonal" nodule, type C, at the right; internodular, sharply defined, angular, compressed lobules of poorly organized, pleomorphic cells, type H, in left half of field. × 255.

FIG. 10. Case 2. Center of proliferation in a "fetal" nodule, type B. × 255.

FIG. 11. Case 2. Internodular, sharply defined, angular, compressed lobules of poorly organized, pleomorphic cells, type H, at the left; nodule of somewhat irregular follicles containing thin, vacuolated colloid and separated by a moderately abundant stroma, type I, at the right. × 255.



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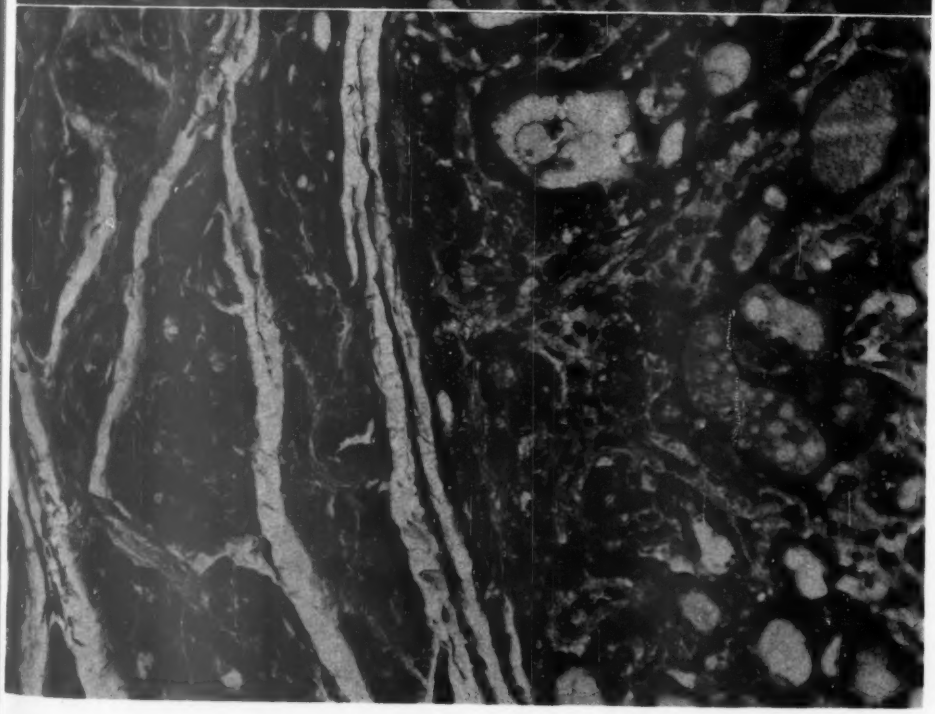

11

CONGENITAL CIRRHOSIS OF THE LIVER WITH KERNICTERUS

Report of Two Cases in Siblings with a Discussion of the Relationship to So-Called Neonatal Hepatitis and to Iso-Immunization Disease *

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A form of cirrhosis of the liver with parenchymal "giant cells," occurring in early life, has been described recently.[1-3] Cases observed weeks or months after birth have been regarded as late manifestations of a process initiated during fetal life; in many of these instances, however, the age at onset of clinical symptoms, including jaundice, is such that postnatal acquisition of the disease is theoretically possible. In a few cases, however, the demonstration of advanced cirrhosis of the liver at or shortly after birth proves conclusively that the disease existed during intra-uterine life (Dible *et al.*[2] and Stokes *et al.*,[4] case 26). The two cases herein reported belong to the latter group.

Terminology currently in use to describe this disease favors such designations as viral hepatitis, fetal hepatitis, and neonatal hepatitis. Incompatibility of blood groups cannot be demonstrated.[3,5] The histologic pattern has been regarded by some to be "highly specific"[2]; others were unable to find clear-cut differences from the hepatic alterations sometimes found in erythroblastosis.[6] There is a need for a more precise characterization of the pathologic anatomy than is currently available.

The two cases, reports of which follow, were of siblings resulting from third and fourth pregnancies; they died 6½ hours and 45 hours after birth, respectively. The observation of kernicterus in one of these babies is the first to be recorded in this disease.

Report of Cases

Case 1 (Mother's Fourth Pregnancy)

The mother, S. S., was 26 years old, para III, gravida IV. Her past history was non-contributory. Neither she nor her husband had ever had syphilis. There was no history of exposure at any time to alcohol, heavy metals, chloroform, or other toxic substances. She had never been jaundiced and had never been transfused. Her first pregnancy had terminated in January, 1950, with full-term breech delivery at another hospital. The infant died 2 days after delivery and post-mortem examination revealed pulmonary atelectasis. The liver was normal histologically. (Sections were reviewed for the purposes of this report.) Her second pregnancy ended in September,

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1950, with a spontaneous abortion at 3 months of gestation. Her third pregnancy terminated at 8 months; the infant expired 6½ hours after delivery (case 2).

The patient conceived again in March, 1952. Because of her history of a spontaneous abortion, she was given stilbestrol, 5 mg. daily. Two episodes of slight vaginal bleeding occurred in May and June, and the stilbestrol was increased gradually to 250 mg. daily. Her blood pressure ranged from 120/60 to 130/90 mm. of Hg. Albuminuria appeared in the fifth month of pregnancy in amounts varying from 1 to 3 plus; this had not been present prior to pregnancy, and disappeared at its termination. There were no casts or red blood cells. There was no edema or jaundice at any time.

On December 4, 1952, she was delivered of a full-term female infant weighing 5 lb., 9 oz. The liquor amnii was not stained. No abnormalities of the infant's heart, lungs, or abdomen were noted. There were no apparent congenital anomalies. Seventeen hours after birth, a small amount of blood-tinged vomitus appeared. Mild icterus and a small purpuric area on the left thigh were then noted. On the day after delivery, the stool contained meconium mixed with bright red blood. The hemoglobin was 12 gm. per 100 ml. (photoelectric colorimetric method). The red blood cell count was 3,500,000 per cmm.; the white blood cell count was 8,000 with 30 per cent polymorphonuclear leukocytes, 46 per cent staff cells, 20 per cent lymphocytes, and 4 per cent monocytes. The platelets were normal. The Coombs test was negative. The blood group was A, Rh positive. There were no chemical examinations of the blood. The infant was given 50 ml. of compatible group A, Rh positive blood and 35 ml. of 5 per cent glucose in distilled water. The next day, her condition seemed to deteriorate. She was lethargic, refused feedings, and regurgitated bloody material. Stools continued to show gross blood. Cyanosis, twitchings, and cerebral cry appeared. The infant died on December 6, 1952, 45 hours after birth.

The placenta weighed 470 gm. and measured 15 cm. in diameter and 2 cm. in thickness. The attached cord measured 42 by 1 cm. and was inserted eccentrically; its macroscopic appearance was normal. The maternal and fetal surfaces showed no significant gross abnormalities. There was a pale yellowish white infarct, measuring 1.5 by 1.0 cm., just beneath the fetal surface near the center of the placenta. Sections revealed many chorionic villi which were two to five times the normal size, with a corresponding reduction of the intervillous spaces. The stroma was moderately cellular and the capillaries were engorged. There were no evidences of inflammation or vascular disease. The trophoblast appeared normal. There were small areas of calcification in the infarct. Most of the capillaries were located near the periphery of the villi; there was no erythropoiesis in the stroma. The villi were completely covered by trophoblast and in general the impression was that of a placenta at a stage of development less than full term. Langhans' cells were not observed. With the Prussian blue reaction, iron-positive material was observed in the cytoplasm of stromal cells of the chorionic villi, and also occasionally near the surface of villi beneath the trophoblast. The placenta was recorded as showing enlargement and immaturity of chorionic villi.

Protocol of Necropsy (Lebanon Hospital no. 2593)

The body was that of a 45-hour-old, white, female, newborn infant. There was a distinct icteric tint in the skin; this was more marked in the sclerae. The body measured 48 cm. in length and weighed 1,375 gm. A slight amount of bloody material was seen at the anal margin. The anterior fontanelle measured 3.5 by 2.5 cm. There was an ecchymosis on the left thigh. The lips were dry, rough, and bright red, with evidence of freshly crusted blood.

The abdominal cavity did not contain an excessive amount of fluid. The umbilical arteries and vein were normal. The inferior edge of the liver was about 1 cm. below the costal margin. The spleen was not seen below the costal margin. Both pleural cavities and the pericardium contained a normal amount of fluid, which was slightly icteric.

Cardiovascular System. The heart weighed 15 gm. (normal, 10 gm.). The foramen ovale was patent. There was a slight icteric tint in the endocardium. The ductus arteriosus was patent; its lumen was tiny. The pericardium, chambers, and valves were normal.

Respiratory System. Both lungs together weighed 50 gm. (normal, 33.7 gm.). The gross appearance on surface and section was normal. A small amount of icteric fluid was present in the bronchi.

Gastrointestinal System. There were two small areas of submucosal hemorrhage in the midportion of the esophagus, each measuring 0.5 cm. At the lower end of the esophagus, just above the cardia, there was an irregular area of dark, hemorrhagic discoloration. There were two round, punched-out ulcers in the mucosa of the stomach; the edges were not raised or indurated. They were surrounded by rings of hemorrhage, 1 to 2 mm. in width. One was situated in the proximal portion of the lesser curvature; the other, in the anterior wall. Both measured about 0.3 cm. in diameter. There was no fresh blood in the stomach. The duodenum contained a tiny, dark red blood clot. The contents of the remainder of the small intestine appeared normal. The large bowel contained dark green meconium. In the mucosa of the rectum, about 1 cm. from the anus, there was an irregular, hemorrhagic ulcer, measuring 0.5 by 0.1 cm.

Hepatobiliary System. The general configuration of the liver was normal, but there was a uniform reduction in size (Fig. 1); the liver weighed 40.1 gm. (normal, 66.3 gm.). It was pale tan and all surfaces were studded irregularly with nodules. These nodules were more or less widely separated; they varied in size from 1.0 to 4.0 mm.; some of them were green, but others had the same color as the surrounding

parenchyma. The nodules on the surface were slightly but distinctly elevated; some of those found on section appeared flat, while others bulged above the cut surface. The areas between the nodules varied in appearance: in many places the surface was smooth and shiny, and in other places a distinct fine nodularity of cirrhotic type was present. On the under surface of the left lobe, this nodularity was coarser and more readily apparent. The consistency of the liver was markedly increased. The capsule was thin and transparent, transmitting clearly the color of the underlying parenchyma and nodules. The cut surface was grayish tan and the normal parenchymal markings could not be made out. There was a rather uniform appearance, with a network of barely visible, very delicate, fine, gray lines. The vessels and intrahepatic bile ducts appeared normal.

The gallbladder was distended with watery, pale yellow bile. The extrahepatic bile ducts, hepatic artery and veins, and portal vein, together with their major subdivisions, were normal. The papilla of Vater was located normally and had a normal lumen which was readily probed.

Spleen. The spleen weighed 13.1 gm. (normal, 4.0 gm.). The capsule was thin and shiny and transmitted the dark red color of the underlying parenchyma. Section revealed dark red, normal parenchyma. The vessels were normal.

Genitourinary System. Each kidney weighed 12.0 gm. (normal, 13.6 gm.). The gross appearance was normal. The ureters, bladder, uterus, and adnexa were normal.

Adrenal and Thyroid Glands. Normal.

Brain and Cranial Cavity. The brain weighed 310 gm. (normal, 226.8 gm.). The external configuration was normal, and the hemispheres were symmetric and equal in size. The basilar artery and hexagon of Willis showed no abnormalities. Multiple coronal sections at the usual levels revealed no significant abnormalities of the ventricular system, brain stem, or cerebellum. The basal ganglia presented a distinct, uniform, and symmetric bright yellow discoloration, typical of kernicterus. These were the only pigmented areas. The tentorium and falx were intact. There was no evidence of hemorrhage. The venous sinuses were explored and found to be normal.

Microscopic Description

Liver. Sections of the liver revealed a striking transformation of the normal pattern (Figs. 2, 3, and 4). The usual lobular architecture could not be made out easily, but examination of sections from differ-

ent lobes revealed essential preservation of the normal lobular structure. The parenchyma of the liver presented a bizarre appearance; it was characterized by multinucleated, syncytial, cytoplasmic masses of extremely irregular size, shape, and distribution. Some of these contained as many as forty or more nuclei and measured roughly 100 μ or more in diameter; others contained two or three nuclei and were no wider than a normal hepatic cord. Cell boundaries were not distinguishable and normal hepatic cords were encountered only occasionally. Careful study of many fields led to the distinct impression that the multinucleated syncytia were continuous with whatever hepatic cords remained (Fig. 6), and that the syncytia did not represent giant cells in the usual sense. Many of the syncytial masses showed multiple vacuoles, some of which contained polynuclear leukocytes, the pattern being that of necrobiotic tissue undergoing dissolution (Fig. 8).

Although most of the parenchyma was transformed in the manner described, nodules of hepatic parenchyma of relatively normal structure still existed (Fig. 5); these corresponded to the circumscribed nodules, some bile-stained, noted on the surface and cut section in macroscopic examination. In these areas, occasional cell boundaries could be made out. The pattern of cords and sinuses was within normal limits, and the general architecture of hepatic lobules was preserved. It was presumed that these areas represented residual hepatic parenchyma, not yet transformed into the bizarre patterns of the liver elsewhere. Furthermore, it was noteworthy that there were histologic evidences of activity of the destructive process, whatever it might be, in these residual nodules; all stages of necrobiosis were present (Fig. 5). Eosinophilic "smudging" of cytoplasm, coarse vacuolation of cytoplasm, pyknosis, necrobiosis and disappearance of nuclei, fragmentation of cells, collapse of framework, and infiltration by polymorphonuclear neutrophils were observed.

In addition to the multinucleated cytoplasmic masses, the sections revealed a very cellular tissue of irregular pattern, which appeared to be composed of collapsed sinusoids, with their lining cells and contained blood and a richly fibrillar connective tissue infiltrated by inflammatory cells (chiefly polymorphonuclear leukocytes). These areas were uneven in distribution and seemed to occur indiscriminately in any portion of the lobule (Fig. 3). Finally, most fields contained aggregates of immature blood cells (Fig. 3), predominantly undifferentiated forms. The degree of extramedullary hematopoiesis in these sections was excessive. Many of the cells were large, with heavy nuclear membranes, sharp nucleoli, and intranuclear clear spaces; the

cytoplasm usually took basophilic stains and the general impression was that of immature, undifferentiated stem cells of the hematic series. Other cells, however, showed evidences of differentiation along erythroid lines. In some areas, the liver cells appeared to have undergone compression atrophy at the expense of clusters or islands of hematic cells.

These hematopoietic foci, when projected three dimensionally by serial examination of many fields, appeared to be extrasinusoidal, and were found in what can best be described as dilated spaces of Disse (perisinusoidal lymph space). The impression was strengthened in sections stained for argyrophilic fibers. There were no observable fibers, membranes, or other tissue components intervening between these cell clusters and the cytoplasmic membrane or exterior of the parenchymal tissue.

The cytoplasm of many of the large multinucleated masses was deeply pigmented, with a finely granular, rich brown pigment (Fig. 4). In addition, many areas of cytoplasm were vacuolated and had a foamy appearance. A similar foamy pattern was seen in the cytoplasm of many mononucleated cells. With polarized light, birefringent crystals were not found. Bile capillaries could not be made out, nor were typical bile thrombi found, except in occasional areas, which corresponded to the circumscribed green nodules described grossly; in some of these nodules markedly distended bile capillaries containing bile thrombi could be observed (Fig. 7). Mitotic figures were not found.

Glisson's capsule was thin, and showed no effect from this profound alteration.

Silver Impregnation of Liver. The argyrophilic reticulum of the liver was well preserved and stained deeply. With the aid of this stain, it became clear that the normal lobular architecture was basically preserved and that the transformation of the parenchyma and collapse of varying portions of lobules to different degrees had taken place on a monolobular basis. The silver stain also helped to define the multinucleated cellular masses as aggregates of hepatic cytoplasm and nuclei, which were continuous with residual hepatic cords (Fig. 6). In the areas of collapse the impression was that of condensation of argyrophilic fibers following disappearance of parenchyma. In other areas the silver stain helped to bring out surviving cords of liver cells which were not so easily recognized in the hematoxylin and eosin stains.

Staining for Lipids. With the Sudan stain, the stainable lipid of the liver was found to be very unevenly distributed. Where present, it

was located in the cytoplasm of multinucleated syncytia, corresponding to vacuolated cytoplasm, in large amounts in the nodules of residual parenchyma, and occasionally in Kupffer cells and free phagocytes.

Elastica-van Gieson Staining of Liver. The vascular system of the liver was normal with the elastica-van Gieson stain. There was a marked increase in collagen fibers throughout all portions of the section.

Staining for Iron. Almost all of the brown pigment in the multinucleated cell masses of the liver gave a positive reaction with the Prussian blue method (Fig. 16). Most of the iron-positive material was confined to the multinucleated cell masses; finer deposits were present elsewhere, particularly in smaller liver cells, some Kupffer cells, and free histiocytes. There was no fuchsin-positive pigment.

Spleen. In sections of the spleen, the follicles were normal in size and distribution. They did not contain germinal centers. The splenic sinusoids were prominent, increased in number, and engorged with blood. In general, the appearance was that commonly associated with chronic portal stasis. The cellular population of the red pulp was not increased. There was no increase in hematopoiesis; there was a slight increase in polymorphonuclear leukocytes. The Prussian blue reaction disclosed very scanty deposits of iron-containing pigment, normal or less than normal in amount, and contained chiefly in the cytoplasm of sinus endothelial cells.

Kidney. The renal glomeruli were normal. The epithelial cells of the proximal convoluted tubules showed marked granular and vacuolar degeneration with "balloon" swelling. In the pyramids, the straight capillaries were intensely engorged with blood. Crystals were not found by examination with polarized light. In sections stained with hematoxylin and eosin, numerous bluish structures, usually vesicular and often containing amorphous material, were found in the vicinity of the proximal convoluted tubules, with respect to which they could not be precisely localized (Fig. 14). The von Kossa reaction was negative; the Prussian blue reaction occasionally was slightly positive. It was presumed that these structures were complex calcium-iron salt deposits. Bile casts were not present. The cytoplasm of the epithelial cells of the distal convoluted tubules contained moderate amounts of Prussian blue-positive granules (Fig. 17).

Pancreas. The islets of Langerhans were increased in size and number (Fig. 11). There were several intralobular foci of inflammation with an infiltrate including polymorphonuclear neutrophils (Fig. 12).

Occasionally, delicate interstitial fibrosis was noted. Stains for iron revealed large amounts in perinuclear aggregates in the epithelial cells of the acini (Fig. 15). Occasional siderophages were present in the interacinous connective tissue. There was no pigment in the islets.

Anal Ulcer. There was an area of erosion of the anal mucosa with marked hemorrhage into the submucosa and acute inflammation.

Stomach. There was an area of erosion of the gastric mucosa with local hemorrhage and inflammation. Section of esophagus revealed similar changes.

Brain. Sections from various portions of the cerebrum, cerebellum, and brain stem revealed no significant microscopic changes. Pigment could not be identified microscopically in the sections of the basal ganglia.

Heart. Sections of the heart revealed moderate amounts of Prussian blue-positive granules in the myocardial fibers.

Lungs. Most of the pulmonary alveoli contained mononuclear cells, chiefly phagocytes. Occasional polymorphonuclear neutrophils also were present. Careful examination of the epithelial lining cells of the bronchial tree disclosed no inclusion bodies. One or two small collections of intra-alveolar squames were noted. There was no hemorrhage.

Bladder, Skin, Cervix, Lymph Node, Tongue, Sublingual Salivary Gland, Pituitary Body, Small Intestine, Spinal Cord, Bone and Bone Marrow, Diaphragm, Ovaries, Thymus, Thyroid and Parathyroid Glands. No significant changes.

Anatomical Diagnosis. Congenital "giant cell" cirrhosis of the liver, with early icterus; kernicterus; congestive splenomegaly; mucosal erosions of esophagus, stomach, and rectum with hemorrhage into gastrointestinal tract.

Iron Content. Quantitative determinations for iron in the liver and kidneys (by Dr. Max Friedman, Chemist at Lebanon Hospital) yielded the following results: liver, 0.729 per cent of dry tissue (normal, 0.228 per cent of dry tissue) and kidney, 0.141 per cent of dry tissue (normal, 0.043 per cent of dry tissue).

Additional Notes on Parents. During the months following the death of this infant, a series of tests of liver function were carried out on the parents. Those on the father were normal. Results of those on the mother were as follows. The cephalin flocculation test was 4 plus on three occasions; the total cholesterol was normal, but the esters ranged between 30 and 35 per cent; and the total serum protein was normal with a reversal of the albumin-globulin ratio. On April 24, 1953, a specimen of the mother's liver was obtained by needle for

biopsy. Microscopically, there was moderate variation in the size of the nuclei of hepatic cells. Occasional nuclei were small and hyperchromatic; others were large and "ballooned." A rare binucleated cell was observed. There was no evidence of bile stasis. The cytoplasm of the hepatic cells was finely granular and stippled with light brown bile pigment. Rarely, a cell showed basophilic smudging of the cytoplasm. There was no coarse cytoplasmic vacuolation or reticulation. The Kupffer cells appeared normal. There was no inflammatory infiltrate. Stain for iron-containing pigment was negative. The diagnosis was liver cell damage,[7] graded 1 plus.

Analysis of the blood groups of the parents was carried out by Dr. Solomon Dresner, hematologist at Lebanon Hospital, and by Dr. Philip Levine at the Ortho Research Foundation. The results are shown in Table I.

TABLE I

Blood Groups and Rh Types of Parents of Siblings (cases 1 and 2)

		D	C	E	c	Rh	Probable genotype
Father	A	+	+	0	0	+	(R_1R_1) homozygous
Mother	A	+	0	+	+	+	(R_2r) heterozygous

The Coombs test on the mother was negative. Tests of both bloods for antigenic components indicated that the mating was incompatible for S, Kell, Le_a, and P, but there were no demonstrable antibodies for these factors.

CASE 2* (MOTHER'S THIRD PREGNANCY)

The mother (same as in case 1) experienced a threatened abortion during the first trimester of this pregnancy and was treated with large amounts of diethylstilbestrol. She delivered uneventfully a male infant on June 29, 1951, at 35 weeks of gestation. The infant's birth weight was 4 lb., 8 oz., and it measured 45 cm. in length. His condition was described as fair at birth, but perioral cyanosis was noted. He was placed in an incubator with oxygen. Râles were found throughout both lungs and considerable areas of atelectasis were noted. Oxygen and suction were used. The infant's condition became progressively worse and he expired 6½ hours after birth.

Abstract of Protocol of Necropsy (Jewish Memorial Hospital no. 51–33)

The body measured 45 cm. in length and weighed 2,550 gm. There was massive subarachnoid hemorrhage in the left cerebral hemisphere, but no gross anatomical change was found in the brain externally or on section. There was no kernicterus. The heart was normal; lungs,

* We are indebted to Drs. R. Hoenig and A. Schwarz of the Jewish Memorial Hospital, New York City, for permission to publish the data from this case.

airless; liver, reddish and firm; gallbladder and bile ducts, normal; spleen, enlarged, congested, and purplish; adrenal glands, kidneys, ureters, and bladder, normal.

Summary of Microscopic Findings

Liver. Section of the liver revealed a marked alteration of the parenchyma, basically similar to that described in detail in the protocol of case 1, with the difference that the amount of extramedullary hematopoiesis was greater (Fig. 9). Many fields revealed such extreme proliferation of immature hematic cells that the multinucleated syncytia appeared to have undergone compression atrophy by clusters of the former (Fig. 10). There was a distinct tendency toward development along erythroid lines (Fig. 10). In summary, the pattern was characterized by the presence of multinucleated, syncytial, cytoplasmic masses of hepatic parenchyma, disappearance of visible cell boundaries, many collapsed areas of parenchyma with total disappearance of liver cells, some increase in connective tissue, pigment, and lipid in the cellular masses, and very marked hematopoiesis. The degree of destruction and transformation of hepatic parenchyma was somewhat less than in case 1, and it was easier to recognize the lobular pattern. Portal fields and central veins were made out readily. The changes appeared to involve all portions of the lobules.

Spleen. Sections of the spleen revealed a normal follicular pattern. The sinusoids were markedly engorged with blood and increased in number. The pattern suggested portal stasis. Hematopoiesis was within normal limits.

Pancreas. The pancreatic islets were increased in size and number. There were foci of interstitial pancreatitis.

Brain. There was marked hemorrhage into the pia arachnoid. A section of cerebrum showed no significant changes.

Kidneys. In the kidneys there was marked hydropic and granular degeneration with "balloon" swelling of the epithelial cells of the proximal convoluted tubules (Fig. 13). There were no calcium deposits.

Lungs. There was severe diffuse pulmonary congestion, with areas of so-called hyaline membrane disease of the newborn.

Heart, Thymus, Thyroid and Adrenal Glands. No significant change.

In this case, material for study for iron-containing pigment could not be obtained.

General Summary of Clinical and Anatomical Data

Congenital cirrhosis of the liver was found in two successive siblings of parents whose bloods were free of demonstrable Rh or ABO incompatibility. Case 1 was a full-term female infant who died 45 hours after birth; case 2 was a male infant, born after 35 weeks of gestation and dying 6½ hours after birth. There was early icterus in case 1. The Coombs test was negative. There were cutaneous and gastrointestinal hemorrhages.

At necropsy, the liver in each infant was reduced in size and showed a finely granular cirrhosis with scattered, coarse, icteric nodules (Fig. 1). After formalin fixation, only the icteric, coarse nodules turned green. Microscopically, the chief findings were retention of normal lobular architecture and a monolobular cirrhosis of the post-necrotic type (Fig. 2). Multinucleated, cytoplasmic syncytia were conspicuous everywhere (Figs. 3 and 4). The parenchymal cytoplasm contained large quantities of iron-positive pigment (Figs. 4 and 16). Interlobular bile ducts were normal.

Extramedullary hematopoiesis was present to an extreme degree in the liver (Fig. 9). The cell type sometimes was very immature; however, there were suggestions of differentiation along erythroid lines (Fig. 10). Many fields gave the impression of parenchymal cell atrophy secondary to proliferation of hematic cells (Fig. 10). There was marked stasis in the bile capillaries in better preserved parenchyma (Fig. 7). Quantitative chemical analysis for iron disclosed an amount more than double that of the normal. There was an increase of Sudan-positive lipid, much of which was found in the multinucleated syncytia. The involvement of the lobules appeared to occur haphazardly in peripheral, intermediate, or central regions (Fig. 3). Unequal involvement of right and left lobes has been reported[3]; this was not observed by us.

The spleen in each instance was enlarged in the manner usually associated with chronic stasis. Extramedullary hematopoiesis in the spleen did not exceed that found in normal controls. There was no increase in iron-positive pigment.

In the kidneys, iron-positive granules were present in the epithelial cells of the distal convoluted tubules (Fig. 17). Quantitative determination of iron revealed more than double the amount in the normal control. In addition, cases 1 and 2 disclosed a nephrotic alteration consisting of granular and vacuolar degeneration and "balloon" swell-

ing of the epithelial cells of the proximal convoluted tubules (Fig. 13). In case 1, numerous structures consisting of iron and possibly calcium were found (Fig. 14).

In the pancreas, the islets were increased in size and number (Fig. 11) and there was some distortion of architecture, with interstitial pancreatitis (Fig. 12). There were large quantities of iron-positive material in the perinuclear zone of the acinar cells (Fig. 15), and small amounts in phagocytes in the interacinous connective tissue.

Nuclear jaundice of the brain was found in case 1; it was not observed in case 2.

There was a small amount of iron-positive material in the myocardial fibers. The remaining organs showed no significant alterations, except for a few hemorrhages.

In the placenta of case 1, the structural pattern of the villi corresponded to that usually associated with erythroblastosis.[8,9] There were perinuclear aggregates of iron-positive material in the stromal cells of the chorionic villi and a tendency to margination of iron-containing granules beneath the trophoblast. There was no iron-positive pigment in sections of the umbilical cord. Sections of the placenta from case 2 were not available.

Discussion

The theory that congenital cirrhosis of the liver with kernicterus is caused by the virus of serum hepatitis or some other as yet unidentified virus has been advanced.[1-3,5,6,10,11] The virus of serum hepatitis is known to remain active in the blood for long periods.[12-14] It has been postulated[5] that the mother carries this virus in actively transmissible form in her blood for years, and that during each pregnancy it is transmitted across the placenta to the fetus. In those cases in which some siblings escaped the disease, it is necessary to assume further that the virus did not affect these infants, or that, if it did, the liver was damaged without lethal results. Abnormal liver function tests in the living siblings have been cited in support of this hypothesis.[10] Abnormal liver function tests have been encountered also in the mothers of affected infants[10]; and such results were obtained in our patient. Evidence of hepatocellular injury in a specimen obtained for biopsy from the mother's liver has not been recorded previously.

It could be maintained that abnormal liver function tests and histologic evidence of hepatocellular injury in the mother argue in favor of the rôle of serum hepatitis (SH) virus because these phenomena are known to occur in the carrier state in individuals who may have no

history of jaundice or hepatitis.[12] The case for SH virus has been strengthened further by the much quoted experience of Stokes *et al.*,[12] who injected one sample of infant's serum from their case XX into five human volunteers and two different samples of mother's serum into two separate groups of five human volunteers each, producing hepatitis in 40 to 60 per cent of the volunteer group after an interval of 74 to 100 days, consistent with the known behavior of SH virus.

While the results leave no doubt that SH virus was carried in the blood of both infant and mother, certain reservations are made regarding the significance of the findings. The sample of infant's blood was taken at age 9 months, and of the mother's blood 9 months and 35 months after the delivery of the affected infants. Jaundice first appeared in the infant at 2 months of age. It is possible, therefore, that the infant's viral infection was acquired after birth. The history with regard to transfusions and injections is not stated. Transmission from mother to infant via accidental contamination and puncture with ordinary household instruments such as needles and pins also is possible.

The mother's abnormal liver function and hepatic cells are not exclusively indicative of the SH virus carrier state. Identical changes occur in a variety of common hepatic diseases. There are, furthermore, rare instances of hepatic damage by estrogenic substances[15]; our patient had received large quantities throughout most of her third and fourth pregnancies.

There is a striking disparity in incidence between the SH carrier state and neonatal "hepatitis." The incidence of the SH carrier state in the general adult population is 0.5 per cent.[16] While this figure has been arrived at by the study of predominantly male donors, it is assumed that the incidence in females is the same. This would mean that one in every 200 newborn infants has been exposed to SH virus during pregnancy. Because of the rarity of neonatal "hepatitis," one would have to conclude that the fetus possesses a degree of immunity to this virus not shared by any other age group.

The final, and perhaps chief objection to the virus theory is based upon the histopathology of neonatal "hepatitis." The fundamental differences from the changes known to occur in viral hepatitis cannot be dismissed with the explanation that the undeveloped state of the liver alters the pattern of response to injury by the SH virus.[3] Sections of the livers from some of the cases of Stokes *et al.* were examined by Mallory and Lucké; they stated that they had not observed such patterns in viral hepatitis in the adult.[17] A similar view has been expressed

by others.[18] Viral hepatitis has been observed very early in life.[19-21] The morphologic features are then entirely comparable to those which occur in the adult. We feel, therefore, that additional evidence of viral etiology, particularly successful repetition of transmission experiments, must be obtained before this theory can be accepted. Gellis, Craig, and Hsia[5] stated that the evidence for the rôle of SH virus is inconclusive at the present time.

The fact that both of the affected siblings in our series were born after pregnancies during which stilbestrol had been administered over a long period, led us to investigate the possibility that we might be dealing with a transplacental hepatotoxic effect of this substance. No support for this hypothesis could be found. There is no clinical experience suggesting damage to the liver of the fetus after administration of this substance to pregnant women, despite widespread use for many years. Finally, there is no record of maternal treatment with stilbestrol in other cases of congenital cirrhosis. It is concluded that the association with stilbestrol in our two cases is coincidental.

The alterations observed in the liver were advanced and it is not possible to form an exact idea of their genesis without the study of intermediate stages. It is necessary to assume that the state of the liver at necropsy was arrived at by the progressive transformation of previously normal parenchyma. The occurrence of areas or islands of relatively normal architecture (Fig. 5), and the observation of what appear to be intermediate stages in the transformation to "giant cell" cirrhosis, constitute the only supports, at present, for this assumption.

The anatomical pattern belongs to the category of post-necrotic cirrhosis. It is suggested that the acidophilic coagulative necrotic changes in the liver cells in the foci of residual normal parenchyma (Fig. 5) constitute the primary and, in this instance, the active and/or recurrent lesion, and that this is followed by collapse of the framework, inflammation, and fibrosis, on a monolobular scale.[22]

The term giant cell may be a convenient designation for the multinucleated syncytia, but does not apply in a strict sense. They are not "cells" at all, but rather cytoplasmic territories containing aggregates of nuclei, these territories being continuous with the remainder of the hepatic laminae of their respective lobules. This conclusion is arrived at from a study of serial and thick sections and is suggested by Elias' descriptions and stereograms of the architecture of the normal hepatic lobule.[23,24] It is noteworthy that wherever the multinucleated syncytia appear, the outlines and cytoplasmic boundaries of normal liver cells cannot be made out (Figs. 3 and 4).

The exact manner in which the multinucleated syncytia are formed is not known. Available evidence indicates that in the fetal and neonatal periods a variety of injuries to the liver may be followed by the appearance of these forms, and the consensus is that these "giant cells" represent a basic pattern of reaction to injury.[1,6] It seems possible to us that the clusters of nuclei may result from the flowing together and congregation of nuclei which have moved from other parts of the hepatic laminae after the disappearance of the cell boundaries[2]; available evidence is against abnormal ploidy.[25] Mitotic figures were not found in our material.

It is not entirely clear whether the multinucleated syncytia belong to a degenerative or regenerative phase. Similar structures, observed occasionally in the adult liver, have been regarded as evidence of regeneration.[3,26,27] On the other hand, the fetal and neonatal material shows evidences of necrobiosis in the syncytia (Fig. 8).[18] It is possible that they represent neither degeneration nor regeneration, but rather an alteration of form, basically a rearrangement, following certain types of injury. Such an interpretation would be consistent with the finding of these bizarre formations in such widely varying conditions in the antenatal-neonatal periods as syphilis,[25] acquired hemolytic anemia,[6] erythroblastosis,[2,28-30] and nutritional deficiency.[31] The anatomical differentiation of these diseases cannot be based, then, upon the "giant cells," which may be common to all, but would require rather a study of the totality of anatomical changes in the liver and other organs.

A completely normal biliary tree in our cases could be traced to the smaller subdivisions in the portal fields. The marginal lobular ductules were somewhat more difficult to discern, although they could be recognized frequently and appeared essentially normal (Fig. 3). Others have described proliferation of bile ducts at the periphery of the lobules.[3]

The bile capillaries could not be seen at all, except in the solitary coarse nodules of residual normal parenchyma, where they were frequently distended and sometimes contained "bile thrombi" (Fig. 7). Most of the solitary nodules containing these dilated bile capillaries were grossly icteric, whereas the rest of the liver was pale tan. After fixation in formalin, the icteric nodules underwent a dark green discoloration; the remaining parenchyma of the liver did not undergo the change of color usually observed in obstructive jaundice. This behavior is consistent with the histologic finding of dilated biliary capillaries only in the icteric nodules, and implies that bile pigment

observed elsewhere, as in the iron-negative pigment in the nuclei of the brain, was of non-obstructive origin (indirect bilirubin). It is noteworthy that the yellow color of the ganglia in kernicterus does not turn to green after fixation in formalin, and that, according to recent data, the correlation of kernicterus with increased serum bilirubin is with the "indirect" and not the "direct" moiety.[32]

It may be assumed that the dilatation of bile capillaries and their plugging with "bile thrombi" is of the type occasionally seen in acute hepatocellular disease. The obstructive phenomena are generally regarded to be the consequences of cellular injury and concentration of bile. Biliary obstruction at the bile capillary level without obstruction of the biliary duct system has long been known to occur. There have been numerous speculations regarding the mechanism; these include concentration of bile by excessive hemolysis,[33] inspissation of bile,[6] and swelling of hepatic cells with pressure on the bile capillaries.[34] The walls of the bile capillaries are believed to be submicroscopic and their structure has not been fully elucidated.[24] It is possible that bile capillary damage may accompany hepatocellular destruction,[2] with secondary distention and "bile thrombus" formation. Certainly, our study disclosed no anatomical evidence of a congenital structural defect as the initiator of these lesions.

The degree of extramedullary hematopoiesis is far in excess of normal. Furthermore, the type of cell concerned is more primitive than that usually found; many of the cells are too undifferentiated to permit identification with the erythroid or myeloid series. Nevertheless, indications of erythroid direction of hematic cell development could be found. The tendency of these cells to occur in a "paracytoplasmic" location, between the sinus endothelium with its lacework of argyrophilic fibers, and the outer wall of the cytoplasm of the hepatic laminae, was noted. The fact that frequent areas may be observed in which dense aggregates of immature hematic cells are approximated to thinned out, excavated areas of parenchyma, suggests that a form of "pressure atrophy" of hepatic cells may result from the excessive number and size of the hematopoietic foci, which compete for the available space with the hepatic cells (Fig. 10). This would constitute an additional source of injury to parenchyma already damaged in the "primary" manner postulated. Similar observations have been made by Zollinger.[9]

Dible *et al.*[3] noted an especial concentration of hematopoietic activity in the "hyperplastic nodules" of liver cells, and postulated the release there of "some substance of importance for successful haematopoiesis."

It may be significant that hematopoiesis seems to be confined to the liver; the spleen, lymph nodes, and bone marrow do not show evidence of excessive proliferation of hematic cells. This pattern is somewhat different from that usually associated with erythroblastosis, in which the activity is often participated in by the spleen, kidneys, lymph nodes, and other organs in addition to the liver.[8,35] Whether this distinction is basic remains to be demonstrated.

The results obtained with the Prussian blue reaction for iron merit special attention. Most of the yellowish brown pigment in the cytoplasm of hepatic parenchymal cells, including the multinucleated syncytia, gave a positive reaction for iron. Additional Prussian blue-positive material was observed in some Kupffer cells and also in free phagocytes. The amounts in the Kupffer cells appeared to be less than in the normal full-term newborn, a significant proportion of whose normal total of 50 ± mg. of hepatic iron is located in Kupffer cells. The distribution of iron-containing pigment in the liver was similar to that in the cases of Dible *et al.*[3] We were unable to demonstrate hemofuscin. There is no reference to stains for iron in the papers by Craig and Landing[2] and Stokes *et al.*[1]

In other organs the pattern of distribution of iron-containing pigment was distinctive. The spleen and lymph nodes contained only minor amounts. This has been noted in other cases.[3] In the pancreas and kidneys, where the deposition was heavy, the pigment was present almost exclusively in the cytoplasm of the acinar cells and distal tubular cells, respectively. In case 3 of Dible *et al.*,[3] heavy siderosis of the pancreas was noted, and there, too, the pigment occurred in the acinar cells; however, some was found also in the interacinar connective tissue.

Dible *et al.*[3] made quantitative analyses for iron on the livers of their cases 3 and 4, and obtained results within normal limits[36] (0.3 and 0.13 per cent of dry tissue, respectively), despite the very large amounts demonstrable histochemically by the Prussian blue reaction. They interpreted this to mean that, because of liver cell dysfunction, iron could not be held in its usual combined organic form in which it reacts negatively to Prussian blue. Chemical analysis of the liver in our case 1 yielded results which do not conform to those obtained by Dible *et al.*[3] The amount of iron in the liver and kidney was 0.729 and 0.141 per cent of dry tissue, respectively. The calculated total amount in the liver was 87 mg., which exceeds the highest normal figures obtained by Smith[37] (50 to 70 mg.) in his series. In a single control case of a full-term infant dying at 48 hours, the results in liver

and kidney were 0.228 and 0.043 per cent of dry tissue, respectively. The ratio of the amount in the liver to the normal control liver was 3.2:1, and of the amount in the kidney to the control kidney was 3.3:1. Iron-containing pigment was found also in the stromal cells of the chorionic villi, in addition to the more usual margination in the subtrophoblastic layer.

While we have been principally concerned with that form of "giant cell" cirrhosis of the liver in which death occurs within hours after birth, it is conceivable that lesser degrees of this disease may be nonlethal and that such infants might survive to develop jaundice and clinical evidences of cirrhosis weeks, months, or years after birth. Perhaps some cases of juvenile cirrhosis of obscure etiology may have originated as congenital "giant cell" cirrhosis.[3,38] It appears wise, for the present, to separate the fetal, postnatal, and juvenile categories, even if they should prove ultimately to be related. Such a separation will simplify, somewhat, the task of grouping and analysing the data. Future observers must be alerted to collect information regarding familial disease in general, rare or unknown blood groups and antibodies, possible familial defects of erythrocytes, transmissibility to human volunteers, biochemical and hematologic studies of living siblings, and iron metabolism of mothers and infants.

Summary and Discussion of the Relationship to Iso-immunization Disease

We have reported the cases of two successive siblings dying a few hours after birth with advanced hepatic disease of the type commonly referred to as "fetal hepatitis." Six similar cases have been selected from the literature (Dible *et al.*[3] and Stokes *et al.*,[4] case 26) for inclusion in this category (Table II). Incompatibility of blood groups could not be demonstrated and the Coombs test was negative. Only one case presented kernicterus (our case 1). (Kernicterus has not been reported previously in neonatal hepatitis. This may be explained, in part, by the fact that most infants died too early or too late for the appearance of this lesion.[2,3,6] Kernicterus does not occur at birth. It is best observed after the first day of life and may disappear in those who survive more than a few days.)[32,39,40]

In previous studies, any relationship of these cases to iso-immunization disease has been excluded on two main premises, namely, the negative serology and the "giant cell" cirrhosis of the liver, the latter having been regarded as not belonging to the pattern of hepatic involvement in erythroblastosis. Dible *et al.*[3] ruled out erythroblastosis

in their series because of the negative serologic findings and the unusual hepatic changes; they noted the differences from the usual features produced by SH virus, leading them to postulate the existence of some other virus. Craig and Landing[2] and Gellis, Craig, and Hsia[5] classi-

TABLE II

Available Clinical Data in 8 Cases of Congenital Cirrhosis of the Liver with Parenchymal "Giant Cells"

In this series, the hepatic lesion is undeniably of intra-uterine origin because death occurred within 3 days after birth. There was no demonstrable incompatibility of blood groups. The cases of Dible *et al.*[3] and Stokes *et al.*[4] were reported as hepatitis of viral origin.

	Gestation	Birth weight	Sex	Age at onset of jaundice	Age at death	Blood groups and Rh type			Siblings		Coombs' test (cord blood)
						Mother	Father	Infant	Previous	Later	
	wks.	*lb.-oz.*		*hrs.*	*hrs.*						
Ehrlich and Ratner, case 1*	40	5–9	F	17	45	A +	A +	A +	Ehrlich and Ratner 2	None	Negative
Ehrlich and Ratner, case 2*	35	4–7	M	None	6½	A +	A +	Not stated	Normal 1	Ehrlich and Ratner 1	Not done
Dible *et al.*,[3] case 1	40	5–8	Not stated	None	48	A +	Not stated	Not stated	Several normal	Not stated	Not stated
Dible *et al.*,[3] case 2	Not stated	5–6	M	5	52	A +	Not stated	O +	One Normal	Not stated	Negative
Dible *et al.*,[3] case 3†	36?	6–4	F	None	10	O +	A +	O +	One died	Dible *et al.* 4	Negative
Dible *et al.*,[3] case 4†	38	7–8	M	None	36	O +‡	A +	O +	Dible *et al.* 3	Dible *et al.*	Not done
Dible *et al.*,[3] case 5†	Not stated	Not stated	Not stated	None	Still-born	O +	A +	Not stated	Dible *et al.* 3,4	None	Not done
Stokes *et al.*,[4] case 26	37	Not stated	F	Not stated	13	Not stated +	Not stated	Not stated	One	None	Not stated

* Siblings.
† Siblings.
‡ Indirect Coombs' test, negative.

fied all of their cases as "hepatitis" on the basis of a "specific" histologic picture, despite the fact that Craig[41] and others[9,28,30] previously had described similar alterations in erythroblastosis and that in three of their cases of "hepatitis" Craig and Landing[2] demonstrated Rh incompatibility. Smetana and Johnson[18] objected to the virus theory on

anatomical grounds, and advanced the hypothesis that a "failure of development of intercellular bile canaliculi" may be responsible for the lesions in the liver.

The failure to demonstrate Rh or ABO incompatibility does not rule out iso-immunization as a factor. Typical morphologic evidences of erythroblastosis (including kernicterus) have been recorded in the absence of Rh or ABO incompatibility.[41,42] Rare, weak, or unknown blood groups may be implicated. The parents' bloods in our cases were found to be incompatible for S, Kell, Le_a, and P, but antibodies could not be demonstrated. The absence of antibodies in the mother's blood argues against incompatibility, but does not exclude this possibility. Her blood had been tested for the first time for antibodies some 9 months after delivery, and the gradual diminution and disappearance of antibodies following delivery is known to occur.[43] Furthermore, it is possible for incompatibility to exist despite a negative Coombs test; such cases have been reported.[44]

Cirrhosis of the liver with parenchymal "giant cells" is a known accompaniment of iso-immunization disease (morbus hemolyticus neonatorum). It may coexist with icterus gravis,[28,45] hydrops fetalis,[30,45] or hemolytic anemia,[28] or occur independently as the so-called fourth form of hemolytic disease of the newborn.[28,45] That certain cases of congenital cirrhosis of the liver without Rh incompatibility belong nevertheless to the category of iso-immunization disease is suggested by the recent report of a case associated with incompatibility of the ABO type.[29]

The descriptions by Zollinger,[9,28] Reiffenstuhl,[29] Craig,[41] and Harris, Andersen, and Day[6] of the microscopic changes in the liver in erythroblastosis follow so closely the findings in so-called neonatal hepatitis that it seems wise for the present to suspend judgment regarding the significance of the negative serologic findings in the latter. In general, our cases, which conform so readily to the descriptions of neonatal "hepatitis," present many of the known distinguishing features of iso-immunization disease. These are (1) the occurrence in siblings after a normal first child, (2) the anemia and jaundice, (3) immaturity of the placenta, (4) cirrhosis of the liver with parenchymal "giant cells,"* (5) dilatation of bile capillaries with "bile thrombi" and hepatocellular necrosis, (6) splenomegaly, (7) increase in the size and number of pancreatic islets,[9,35,42,46] and (8) marked hematopoiesis along erythroid lines in the liver.

The distribution of heavy deposits of Prussian blue-positive pig-

* Zollinger's[28] excellent account of this hepatic lesion in erythroblastosis seems to have escaped the attention of American and British observers.

ment in the parenchymal cells of the liver, pancreas, and kidneys, with scanty or absent deposits in the spleen, differs from that usually found in erythroblastosis; nevertheless, this pattern has been clearly described in proved cases of erythroblastosis due to Rh or ABO incompatibility.[29,30] The "balloon" swelling of the epithelium of the proximal convoluted tubules and the Prussian blue-positive "bodies" in the kidneys were noted also by Reiffenstuhl[29] in his case of ABO incompatibility and by Zeitlhofer and Speiser[30] in their case of Rh incompatibility.

The discovery of kernicterus in our case 1 would seem to provide an important link in the relationship to iso-immunization disease, inasmuch as kernicterus in the full-term infant is known to occur almost exclusively in iso-immunization disease.[47]

Conclusions

Existing evidence is insufficient to support the theory of viral causation of so-called fetal or neonatal "hepatitis."

Our findings in two cases in siblings indicate a relationship to iso-immunization disease. Kernicterus was present in one case.

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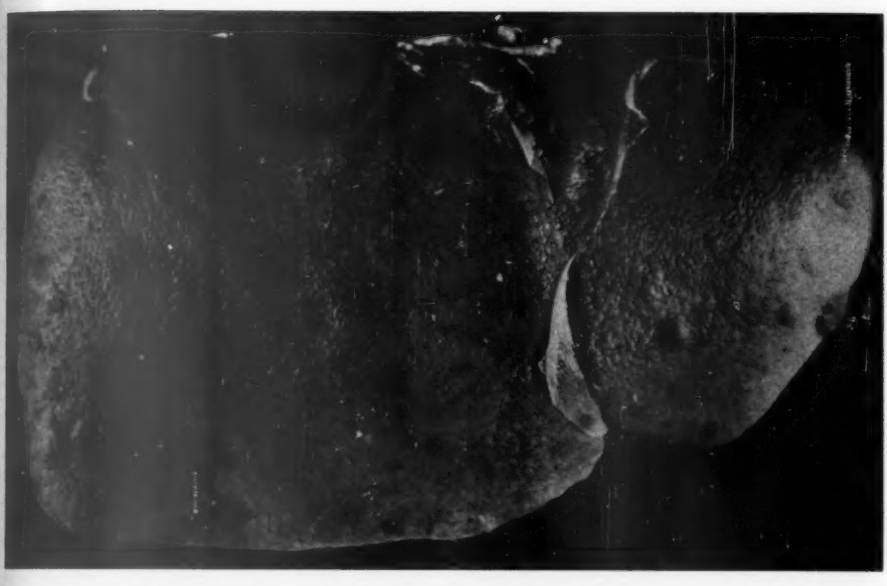
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Case 1. Superior surface of liver (fixed specimen). Coarse and fine granularity with isolated nodules. Reduction in size and weight.

FIG. 2. Case 1. Section of liver showing essential preservation of normal lobular architecture despite marked monolobular cirrhotic transformation. Portal triads and central veins in normal arrangement. Nodularity of surface at left may be noted. × 32.



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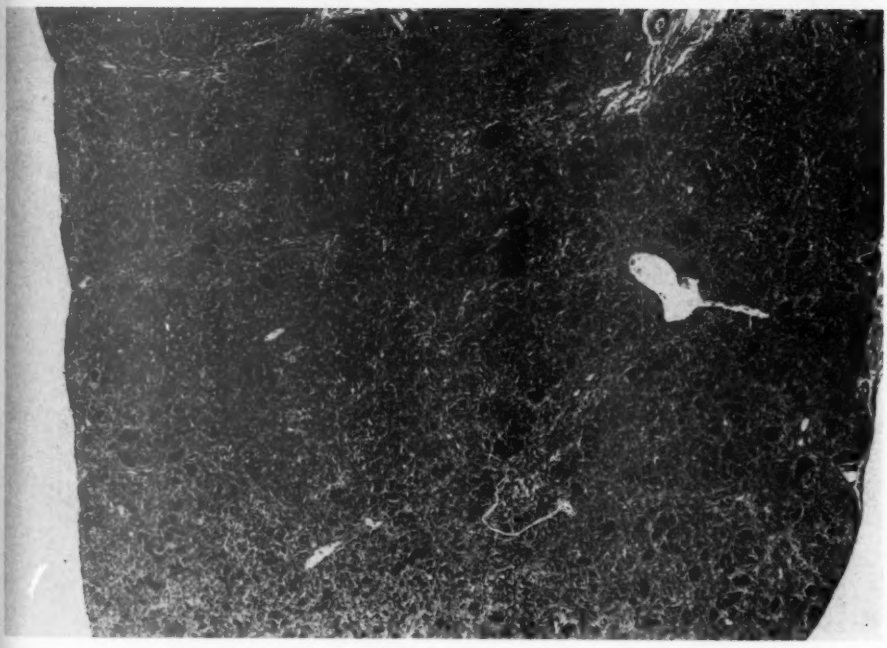

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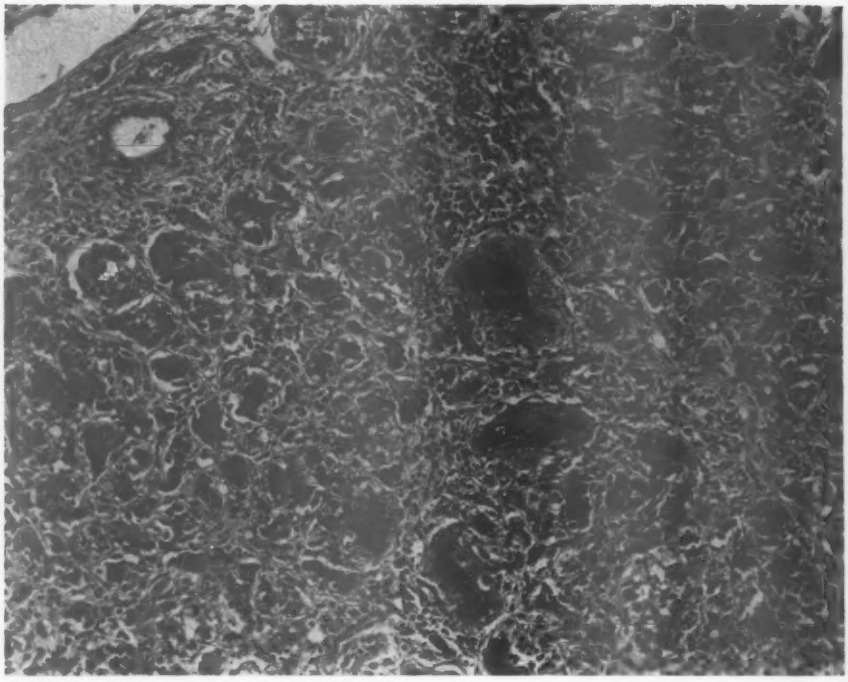
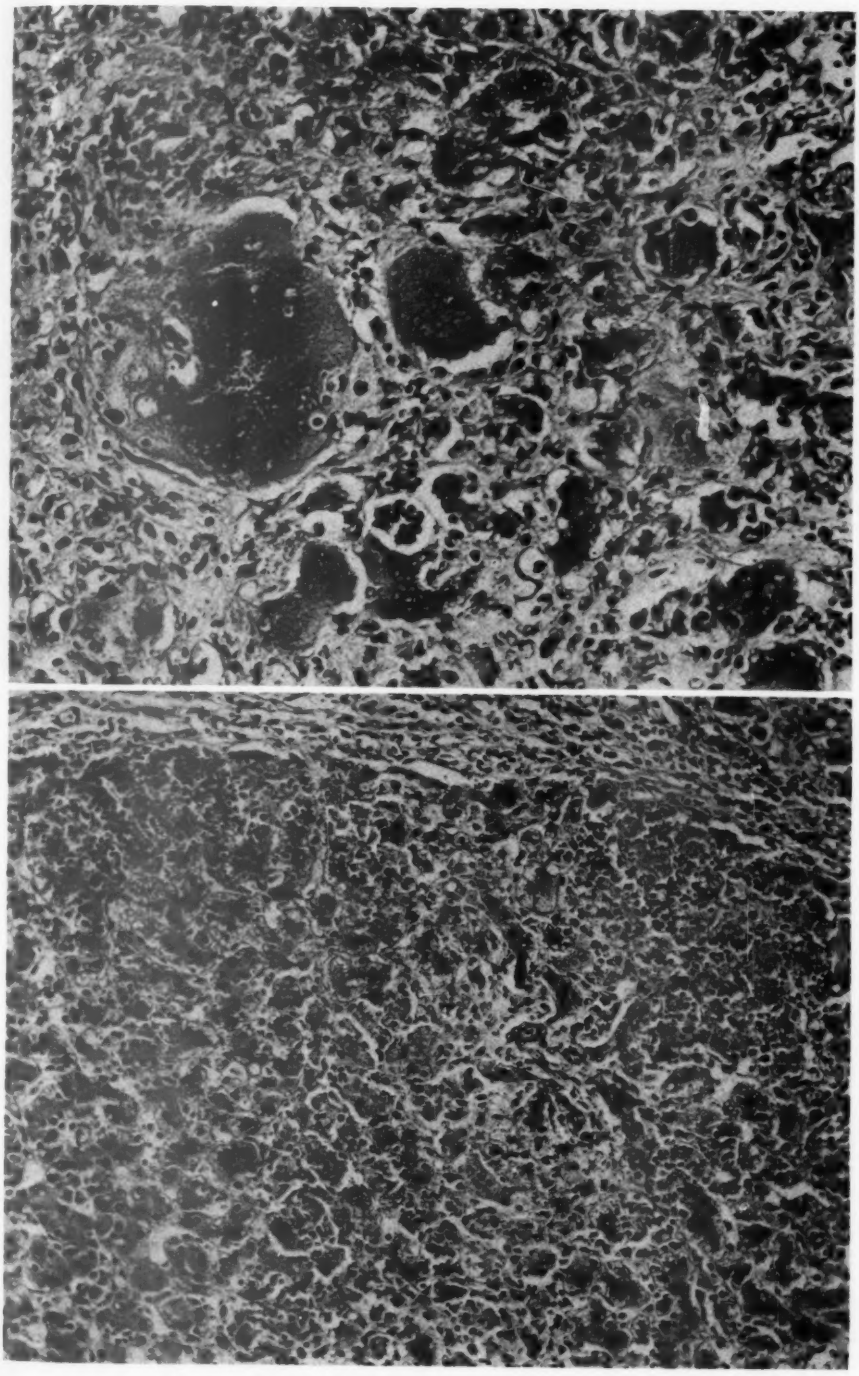


FIG. 3. Case 1. Section of liver showing peripheral portion of lobules and portal triad. Transformation to "giant cell" cirrhosis involving intermediate and peripheral zones of lobules. Multinucleated syncytia and marked hematopoiesis. Normal artery, vein, and duct at upper left. Broad areas of post-necrotic collapse, inflammation, and fibrosis. Hematoxylin and eosin stain. × 180.

FIG. 4. Case 1. Section of liver showing large syncytia, one of which contains more than fifty nuclei. Dense aggregates of granular iron-containing pigment in central portion of syncytium. Hematopoiesis and post-necrotic cirrhosis. Hematoxylin and eosin stain. × 260.

FIG. 5. Case 1. Section of liver showing edge of isolated nodule of "residual" liver. Extensive areas of coagulative acidophilic necrosis of parenchymal cells. Foci of hematopoiesis. Infiltration by inflammatory cells, chiefly polymorphonuclear neutrophils. Hematoxylin and eosin stain. × 180.



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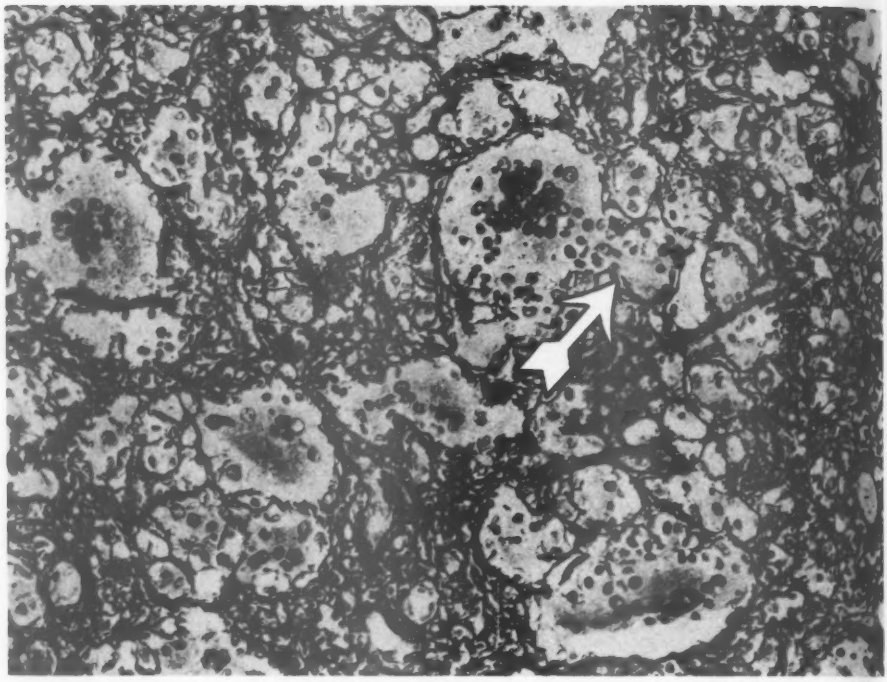
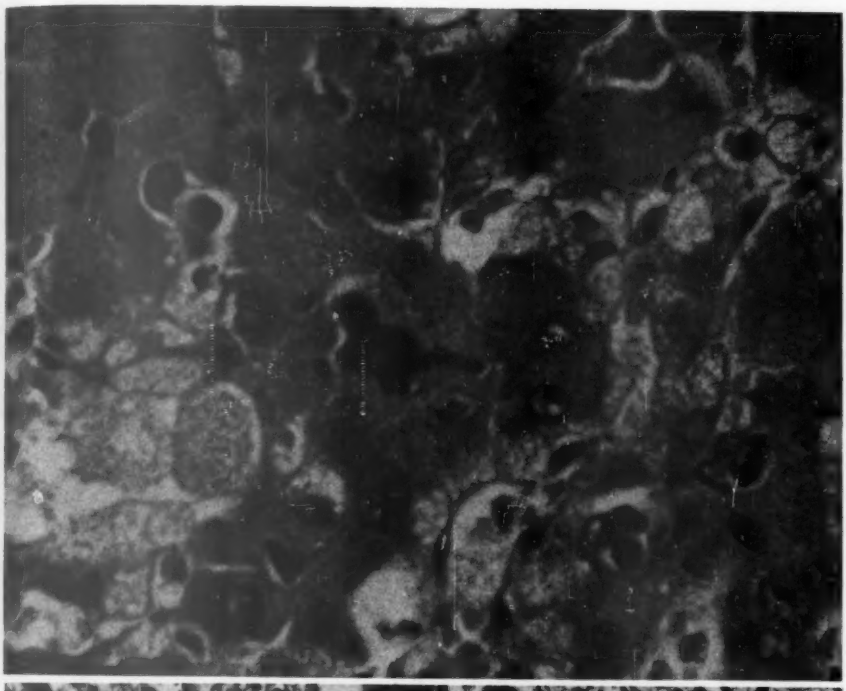


FIG. 6. Case 1. Section of liver. Multinucleated syncytia lying in afibrillar cavities. Continuity with hepatic lamina at white arrow. Broad areas of dense fiber collections indicating collapse of framework. Silver impregnation. × 260.

FIG. 7. Case 1. Markedly dilated bile capillaries filled with "bile thrombi." Hematoxylin and eosin stain. × 520.

FIG. 8. Case 1. Section of liver. Large multinucleated syncytium undergoing degeneration, with invasion by polymorphonuclear neutrophils. Hematoxylin and eosin stain. × 260.



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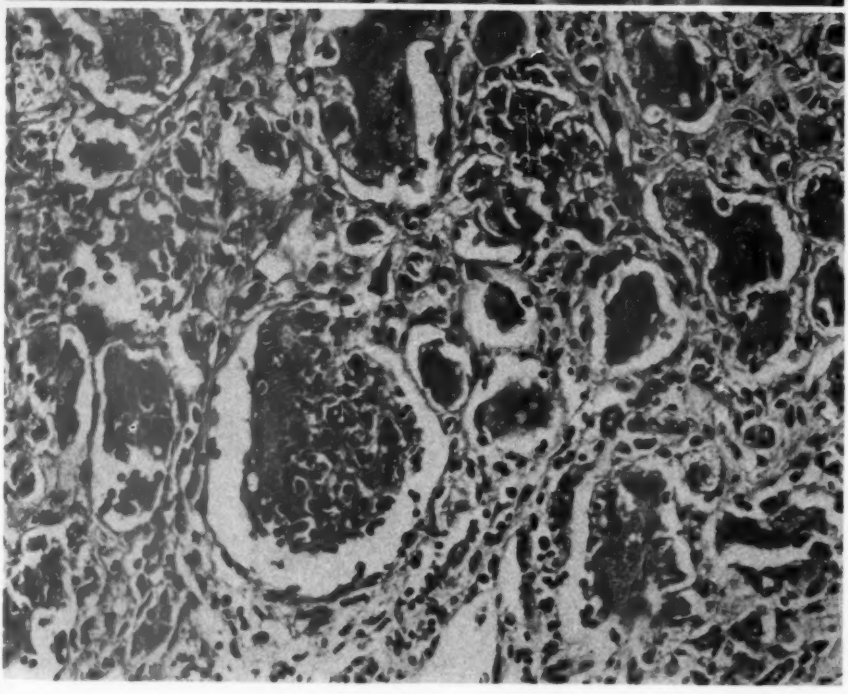

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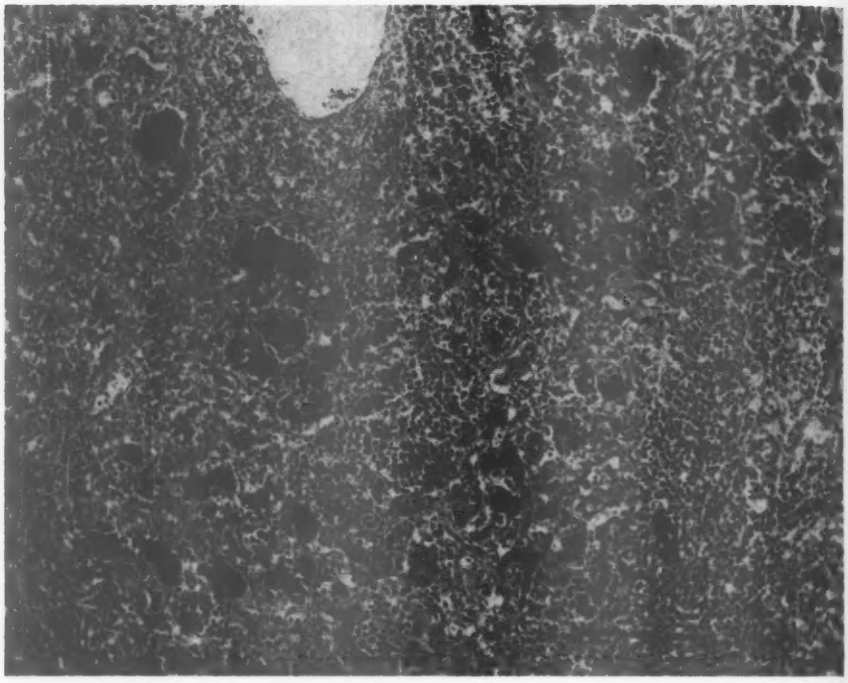
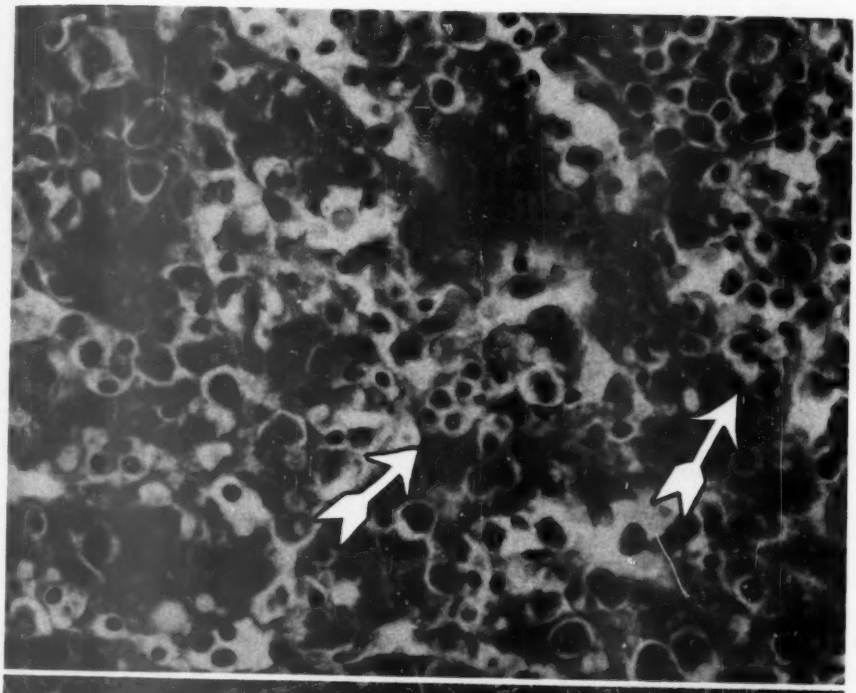


FIG. 9. Case 2. Section of liver to illustrate the degree of extramedullary hematopoiesis. Typical area of "giant cell" cirrhosis. Hematoxylin and eosin stain. × 180.

FIG. 10. Case 2. Mid-portion of liver lobule, showing multinucleated syncytia and portions of hepatic lamina. At upper right, there is a collection of very immature hematic cells. In the center, and scattered elsewhere, are numerous nucleated elements of the erythroid series. Of note are "compression" effects on cells of the hepatic lamina at white arrows. Hematoxylin and eosin stain. × 520.

FIG. 11. Case 1. Section of the pancreas showing increase in size and number of islets of Langerhans. There are at least nine islets in this field. Hematoxylin and eosin stain. × 180.



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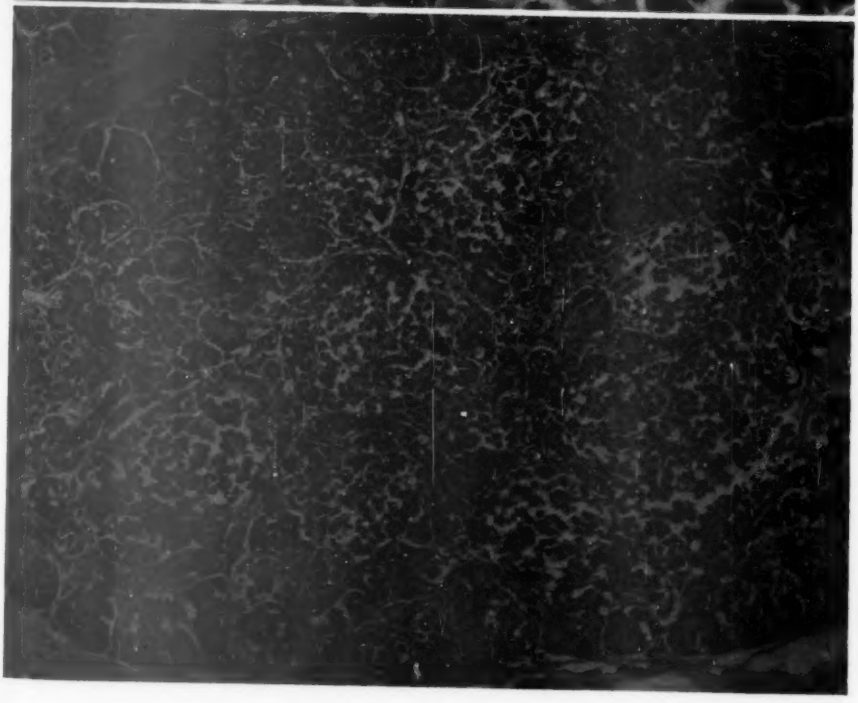

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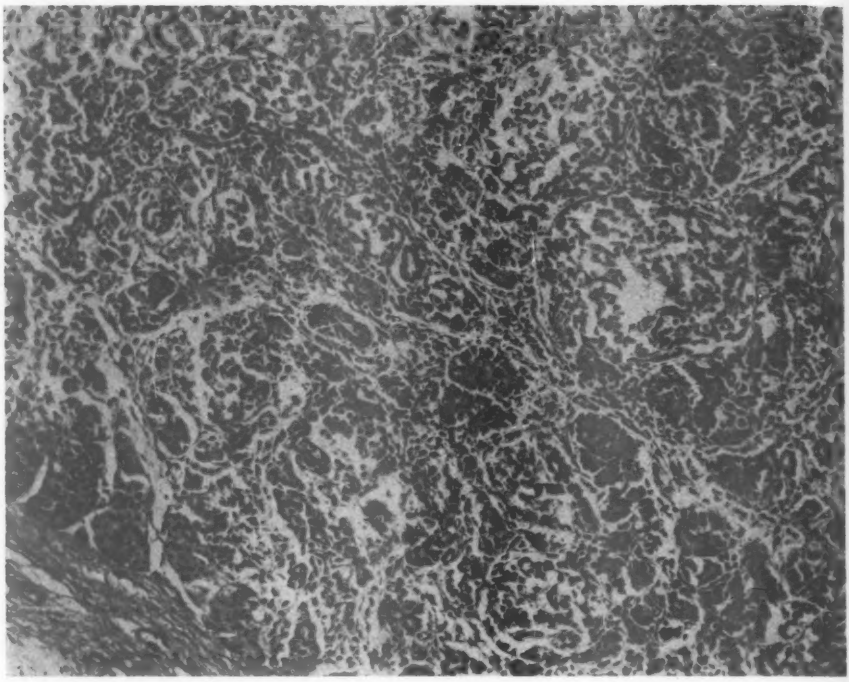
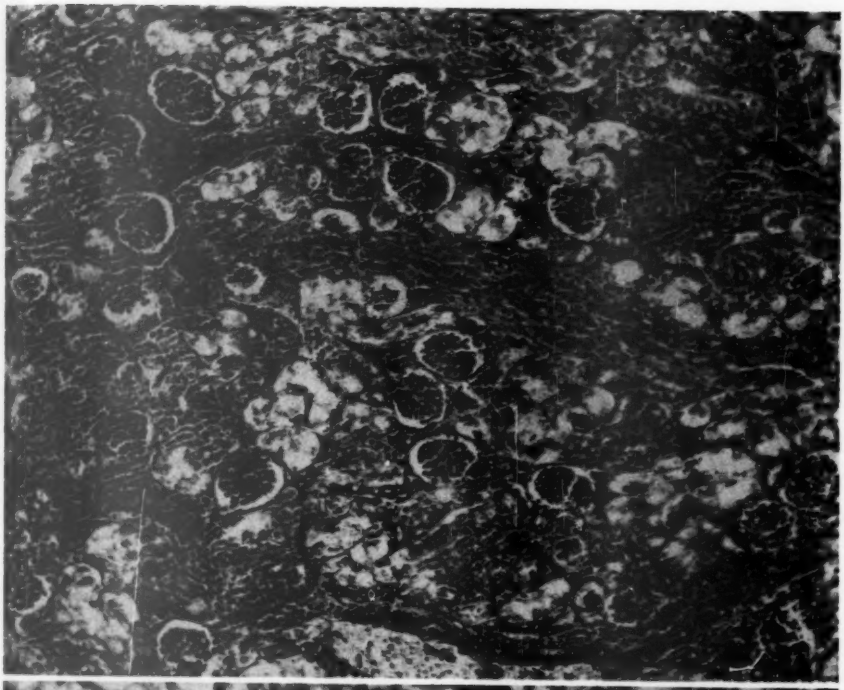


FIG. 12. Case 1. Section of pancreas showing interstitial pancreatitis and fibrosis. Numerous polymorphonuclear neutrophils are present in the infiltrate. Hematoxylin and eosin stain. × 180.

FIG. 13. Case 2. Section of kidney. Marked vacuolar and granular degeneration with "balloon" swelling of epithelium of proximal convoluted tubules. Hematoxylin and eosin stain. × 180.

FIG. 14. Case 1. Section of kidney. One of numerous structures (white arrow) with dark blue outer membrane, vesicular space, and amorphous central material, the latter sometimes Prussian blue-positive. Hydropic swelling of epithelium of proximal convoluted tubules. Hematoxylin and eosin stain. × 520.



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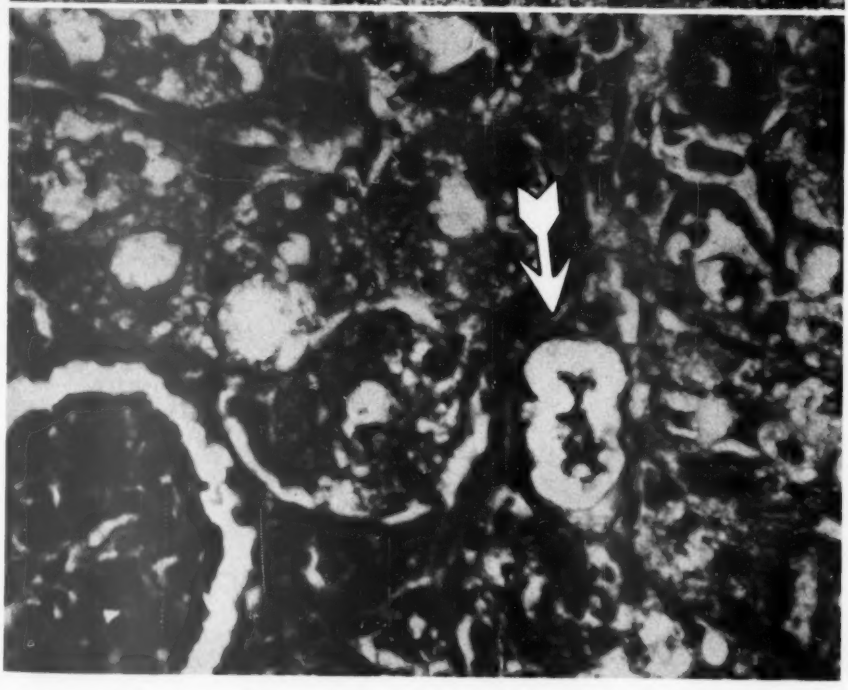

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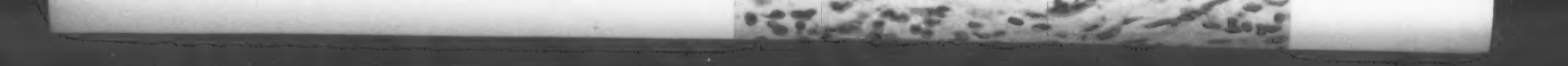
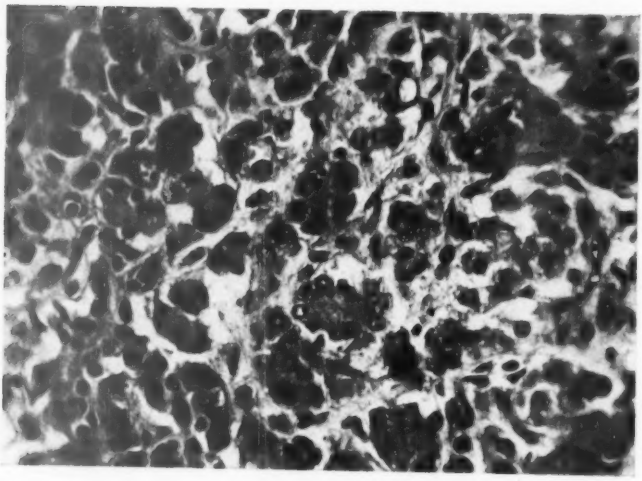
FIG. 15. Case 1. Pancreas. Iron-positive pigment in cytoplasm of acinar cells. Prussian blue-safranin stain. × 320.

FIG. 16. Case 1. Liver. Multinucleated cytoplasmic syncytium (parenchymal "giant cell") with heavy accumulation of iron-positive pigment. Prussian blue-safranin stain. × 280.

FIG. 17. Case 1. Kidney. Iron-positive pigment in cytoplasm of epithelial cells of distal convoluted tubule. Prussian blue-safranin stain. × 320.





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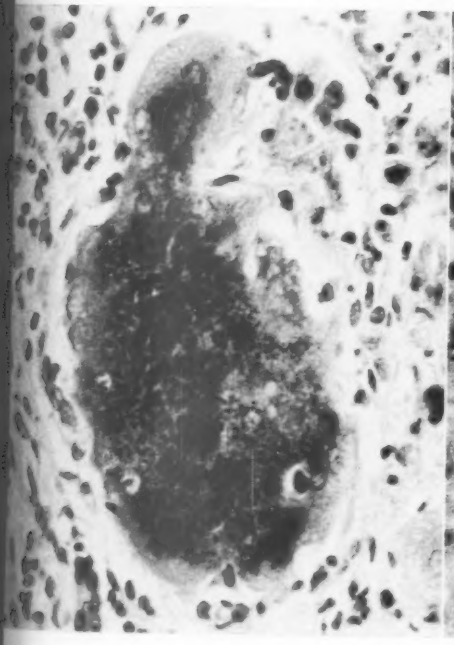

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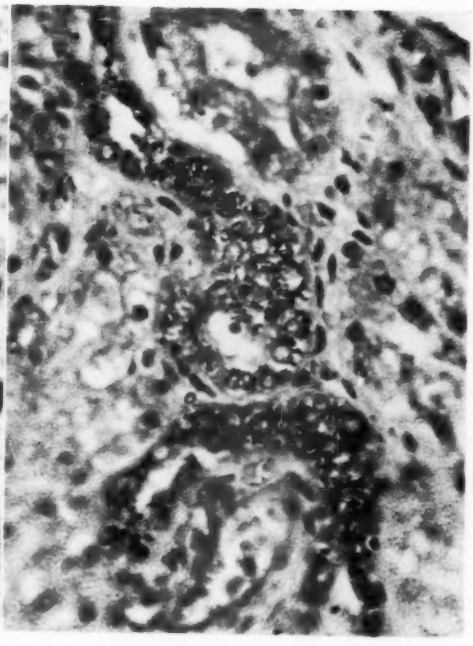

17

MORPHOLOGIC VARIATION IN TISSUE OF THE ORGANISMS OF THE BLASTOMYCOSES AND OF HISTOPLASMOSIS *

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Several investigators over the past few years have reported observations on the parasitic and saprophytic forms of the yeast-like organisms causing the blastomycoses and histoplasmosis. This paper has been prepared to emphasize some of these findings and to confirm and establish some rare occurrences.

The organisms referred to as yeast-like are usually diphasic. In tissue they reproduce generally in the form of budding, yeast-like cells. In artificial or synthetic media exposed to room temperature they will develop filaments and various cell structures. There are several genera which are classified as yeast-like organisms. In the present paper only the following will be considered: *Blastomyces (Zymonema) dermatitidis* (North American blastomycosis); *Paracoccidioides (Blastomyces) brasiliensis, P. tenuis, P. cerebriformis* (South American blastomycosis); *Cryptococcus neoformans* (cryptococcosis or European blastomycosis); and *Histoplasma capsulatum* (histoplasmosis).

In order to simplify this report, the subject matter will be presented under three headings: small and large forms of cells in tissue, occurrence of filaments in tissue, and multiple budding *in vivo* and *in vitro*.

SMALL AND LARGE CELLS IN TISSUE

In the parasitized host, the yeast-like organisms may be seen as either small, young cells or as large, adult forms. These cells are intermingled and are considered to be phases in the parasitic life cycle of the organism.

Since Manwaring's[1] report of the small forms of *B. dermatitidis* in tissue (previously observed by Wade[2]), much more attention has been paid to the tissue forms of the yeast-like organisms. In South American blastomycosis, I[3] observed and reported small forms in 1938, as have other workers before and since that time (Carini,[4] de Souza Campos and de Almeida,[5] Mazza, Niño, and Nicolini,[6] Redaelli and Ciferri,[7] and de Almeida and da Silva Lacaz[8]). The cells of the various species causing this disease reproduce in tissue by multiple budding, as well as by simple budding of small, ovoid, spherical or elongate

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(bacillary) spores (Figs. 4, 5, 11, 12, and 13). In some instances, however, the small forms remain small and appear not to reach the size of the commonly observed mature, large cells (Fig. 3). The small forms of *B. dermatitidis* likewise have been noted on a few occasions and these, like the South American organisms, also tend to reproduce as small cells (Fig 1), yet will be found as the common, large forms in other parts of the tissue (Fig. 2). Very often both types will be found intermingled in the same tissue section. When cultured on artificial media, however, apparently both the small and large cells develop colonies with the characteristics of the one species, so that there is no doubt of the classification of either form.

With *H. capsulatum* the reverse in cell size seems to be true. The common form of this organism in tissue is a small, yeast-like, simple budding, ovoid cell measuring approximately 2 to 4 μ in long axis (Fig. 6). On rare occasions, large forms have been found which in some instances caused confusion in diagnosis. Perhaps the first observation of the large cells, although not actually confirmed, and doubted by some, was by Crumrine and Kessel,[9] in 1931, who described the large forms as being extracellular. The first confirmed report of the large cells in tissue as being *H. capsulatum* was by Hansmann and Schenken[10,11] who should rightfully share with De Monbreun[12] the honor of being the first to cultivate *H. capsulatum* on artificial media. Hansmann and Schenken observed the large forms in thick, red, hard, scaly skin nodules. I studied this organism and considered it to be a new species. I placed it with *H. capsulatum* in the genus Posadasia, calling it *P. pyriformis*.[13,14] This strain since has been considered to be identical with *H. capsulatum* by several workers although it showed large forms in tissue and numerous piriform tuberculate cells (chlamydospores, stalagmospores, or possibly degenerate asci) in culture. In 1943, in a paper with Jorstad, I[15] described and illustrated large forms of this organism in prostatic tissue of an unreported case (Fig. 7). At necropsy, the lungs and prostate revealed lesions. The predominant organisms in the lungs were the usual small forms of *H. capsulatum* whereas in the prostate there were noted the combined small and large cells and areas of exclusively large organisms. In 1947, de Almeida and da Silva Lacaz[16] observed large cells of *H. capsulatum* in the skin of two patients.

In September, 1953, Schwarz[17] unexpectedly found giant forms of *H. capsulatum* in a lymph node from a patient with histoplasmosis, which had been planted on blood agar and incubated at 37° C. A series of explants of tissue from animals experimentally inoculated

with *H. capsulatum* enabled Schwarz to trace the development of the large forms from the usual small cells. This is an interesting observation. Yeast-like organisms, however, very often will develop large forms in the presence of tissue or body fluid and oxygen. This is especially evident in old necrotic lesions, areas of liquefaction necrosis, and in granulation tissue. In tissue explants grown on media the organisms very often will produce giant forms. This is considered to be an attempt to develop into the saprophytic phase. De Monbreun[12] has demonstrated large forms when *H. capsulatum* was grown in sealed serum cultures at 37° C. Schwarz suggested that enzymatic processes might liberate substances in the tissue which act either to stimulate growth or to antagonize factors in viable tissue which might be inhibitors.

In 1953, Vanbreuseghem[18] described the large forms of *H. capsulatum* as belonging to a new species, *H. duboisii.* He stated: "As a matter of fact, a careful study of the literature, as well as personal correspondence with my friend J. T. Duncan, has revealed that the same parasite has probably been encountered several times and always in cases of African origin." Unfortunately, Vanbreuseghem's study of the literature apparently did not include the American publications or he would have found several in which large forms were described and which antedated most of the observations reported in the African literature. He would have noted, also, that the large forms and the cultures they produce have been carefully studied and are now considered to belong to *H. capsulatum.*

The significance of the small (micro-) and large (macro-) forms of both *B. dermatitidis* and *H. capsulatum* is of extreme importance. The confusion of the microforms of *B. dermatitidis* with the usual forms of *H. capsulatum* and the macroforms of *H. capsulatum* with the common forms of *B. dermatitidis,* in tissue, is well recognized. The need for cultures, where these cells occur, therefore, becomes a necessary diagnostic procedure. This was pointed out by Schwarz[17] and by Weed.[19] It was made clear, also, in the publication by Tuttle, Lichtwardt, and Altshuler,[20] who found both small and large forms in a case of systemic North American blastomycosis. Finally, Layton, McKee, and Stamler[21] found both *B. dermatitidis* and *H. capsulatum,* proved by culture, in a case which terminated fatally. This case certainly serves to emphasize the need for culturing the infected tissue.

In the absence of cultures, however, the danger of misdiagnosis can be greatly minimized by careful attention, first, to the cytologic details of the organisms, since they differ from each other in size, shape, man-

ner of budding and cellular detail; and, secondly, to the histopathologic features of the diseases. The latter have been well described and illustrated in the literature and need not be repeated here.

Filamentation in Tissue

Another important morphologic variation in tissue is the presence of filaments or germ tubes. Species of Candida not uncommonly form filaments in parasitized tissue. These hyphal elements are analogous to those seen in culture and are called pseudomycelia. With other yeast-like organisms, such as *B. dermatitidis*, *Sporotrichum schenckii*, and *C. neoformans*, this phenomenon in tissue has not been reported.

C. neoformans in tissue is considered by many as a true blastomycete since it develops as a simple or budding, ovoid or spherical, thick-walled cell measuring approximately 3 to 15 μ or more in diameter. A wide capsule generally surrounds the organism in tissue. In culture, *C. neoformans* forms simple or budding cells similar to those seen in tissue but somewhat smaller on the average. The capsule tends to disappear on subculturing. Hyphae are not formed in cultures. On occasion, in primary cultures, the cells may produce characteristic germ tubes which are lost in subcultures. It is generally stated that hyphae or germ tubes are never found in tissue. In an unreported case of systemic cryptococcosis, cells of *C. neoformans* with germ tubes similar to those seen in primary cultures were noted particularly in areas of necrosis in the liver (Fig. 8). Since evidence of this occurrence could not be found in the literature, this may represent its first observation in human tissue.

Filaments of *H. capsulatum* in human tissue were observed for the first time by Humphrey[22] when he reported their occurrence in a pulmonary nodule. At that time there was some hesitancy in making a definite diagnosis of Histoplasma filaments in tissue, although the mycelial elements had many of the features characteristic of *H. capsulatum* filaments in artificial media.

Schwarz[17] stated: "The arthrospore-like forms described by Humphrey may represent some fungus other than *H. capsulatum*." Because there may be others who share Schwarz's opinion concerning these filaments, it is believed that a second incident of this occurrence should be reported.

In 1939, prior to the examination of Humphrey's slide of lung tissue, sections of an endocardial vegetation from a 53-year-old white man (unpublished case of Rafael Dominguez and Alfred Golden from St. Luke's Hospital, Cleveland, Ohio), were submitted for diagnosis. The

tissue showed numerous yeast-like cells of varying size (Fig. 9) and areas of apparent liquefaction, within which were masses of filaments (Fig. 10). In 1939, our knowledge of the varied structure of Histoplasma *in vivo* was not well developed. No definite diagnosis could be presented at that time. Because of the absence of typical reticuloendothelial histiocytes, which are considered to be characteristic, and the presence of filaments, it was believed that this might be an unusual yeast-like infection, possibly a moniliasis. The slides of this lesion were subsequently reviewed and compared with those of other organs, including the pharynx, where the picture was typically that of histoplasmosis. An atrio-aortic abscess which communicated with the surface of the aortic valve cusp also contained many yeast-like organisms identical with those seen in the vegetation. The filaments, on comparison with those of *H. capsulatum* on artificial media, proved to be identical and it was then considered that the diagnosis of histoplasmosis was definitely established. The substance of the vegetation, devoid of structure, actually was serving as a culture medium. From the standpoint of time, therefore, this was the first observed case of histoplasmosis with filaments *in vivo*. According to the sequence of publication, however, it represents the second case in a human.

In 1952, Haley[23] injected the yeast form of *H. capsulatum* into mice. Thirty days later the mice were killed. One of the animals showed a friable liver, in a necrotic area of which were masses of hyphae and many tuberculate cells characteristic of the saprophytic forms of *H. capsulatum*.

Multiple Budding

The problem of multiple budding has been brought to the fore by several publications. Of the pathogenic fungi, the only organisms known to produce multiple budding in tissue with regularity are those causing South American blastomycosis[3] (Figs. 11, 12, and 13). In experimentally inoculated animals and in varied culture media, many of the yeast-like organisms may be induced to produce multiple budding forms.

The species of Paracoccidioides will form multiple buds at 37° C. in various media, including beef infusion glucose agar, blood agar, and on rice, as shown by Dowding.[24] De Monbreun[25] has described multiple budding in cultures of *B. dermatitidis* made from experimentally inoculated monkeys. Salvin[26] demonstrated multiple budding in *S. schenckii* by growing the organism in YP medium, a fluid peptone medium especially devised for forming the yeast-like phase of *H. cap-*

sulatum. *C. neoformans* will occasionally reproduce *in vitro* with more than one bud. Emmons[27] has demonstrated multiple budding in *C. neoformans* in brain tissue of mice experimentally infected with a strain of organism isolated from the soil.

During the course of the investigation of the effect of fluorides on fungi, it was found that sodium fluoride (1:1,000 to 1:100,000) (Fig. 15) and sodium silicofluoride (1:10,000 to 1:100,000) (Fig. 14) induced multiple budding in both *Candida albicans* and *Saccharomyces cerevisiae*. These forms were more abundant in lower dilutions and became fewer as the dilution of the fluorides increased.

According to Dowding,[24,28] the tuberculations seen on the cells formed by *H. capsulatum* in culture (tuberculate cell, chlamydospore, stalagmospore) are actually spores and the cell itself is termed a macroconidium. Her interpretation of the tuberculations is that they are secondary conidia analogous to the microconidia of the dermatophytes and that these arise from the macroconidia. The chief objection to the use of the term macroconidium is that that structure is generally considered to be multicelled and does not produce buds or secondary conidia.

Sections of whole cultures of *H. capsulatum* embedded in celloidin show that the tuberculate cell is made up of a thick wall from which the tuberculations extend. The projections are short or long, finger-like, bladder-like, or they may show constricted tips. They show no definite contents and are considered to be hollow. No definite connection could be observed between these projections and the inner contents of the cell. Negroni[29] showed that by pressure the verrucous epispore (outer wall) became detached from the inner wall of the structure which he terms a hypnospore. He could find no connection between the two walls. Budding spores form a definite channel to the inner contents of the mother cell.

The conversion of hyphae and tuberculate cells to yeast-like cells was demonstrated by me[30] in 1941 by inoculating the cultured organism on the chorio-allantoic membrane of developing chicks. The hyphae form arthrospores which round up and act as yeast cells when set free. According to Dowding,[24,28] the tuberculations enlarge, become spherical, detach themselves from the parent cell, and act as yeast cells. On the chick membrane, however, the cell wall and the tuberculations disintegrate and the yeast cells form around the chromatin material within the cell. These yeast cells assume the appearance of a mulberry-like cluster. The tuberculate cell behaves more in the manner of a reproductive structure such as an ascus or a sporangium. When the

tuberculate cell is placed on proper medium, De Monbreun,[31] on many occasions, has noted at least six germ tubes arising from within the cell.

Conclusions and Summary

This paper was written in an effort to clarify further and to substantiate some of the phenomena observed in association with the growth and development of certain pathogenic, yeast-like organisms both *in vivo* and *in vitro*. Small and large forms of *Blastomyces dermatitidis, Paracoccidioides (Blastomyces) brasiliensis, P. tenuis, P. cerebriformis,* and *Histoplasma capsulatum* are known to occur in tissue. Cultivation of the organism on artificial media is stressed as a means of differentiating these organisms and thus arriving at a correct diagnosis. The large forms of *H. capsulatum* in tissue are not considered a new species, *H. duboisii* (Vanbreuseghem), but rather are concluded to be tissue variants of *H. capsulatum*.

The occurrence in tissue of filaments of the yeast-like organisms, *B. dermatitidis* and *Cryptococcus neoformans,* has not been reported previously. Cells of *C. neoformans* with germ tubes similar to those seen in primary cultures of the organism were demonstrated in the liver of a patient with systemic cryptococcosis. This perhaps represents the first published report of this occurrence. Filament formation *in vivo* by *H. capsulatum* also was demonstrated in an endocardial vegetation from a previously unpublished case of Dominguez and Golden.

Multiple budding, a common phenomenon with the organisms of South American blastomycosis both in tissue and on certain culture media, may be demonstrated *in vitro* with other organisms, including *B. dermatitidis, Sporotrichum schenckii,* and *C. neoformans*. When grown in media containing either sodium fluoride (1:1,000 to 1:100,000) or sodium silicofluoride (1:10,000 to 1:100,000), *Candida albicans* and *Saccharomyces cerevisiae* also will form multiple buds. The tuberculations seen on the large cells in culture of *H. capsulatum* are considered to be functionless structures and not spores forming yeast-like cells.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

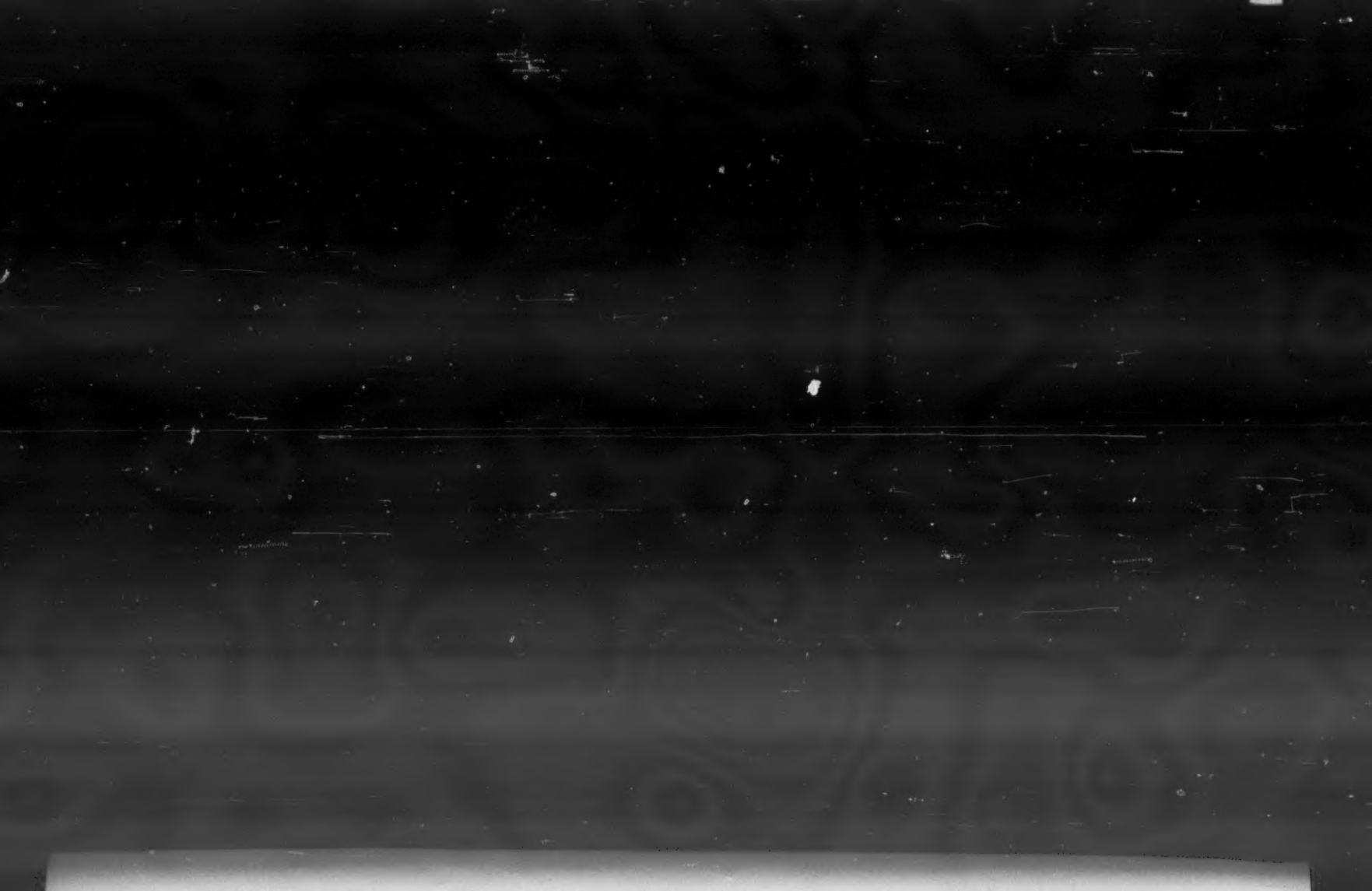
FIG. 1. Small forms of *Blastomyces dermatitidis* within giant cells in sections of skin. Hematoxylin and eosin stain. × 1,170.

FIG. 2. Usual form of *B. dermatitidis* within giant cell in skin. Hematoxylin and eosin stain. × 960.

FIG. 3. Small forms of *Paracoccidioides cerebriformis* within giant cell in skin. Hematoxylin and eosin stain. × 980.

FIG. 4. Mixed forms of *P. brasiliensis* showing single and multiple budding and small and large forms. Hematoxylin and eosin stain. × 1,010.

FIG. 5. Varied sizes of *P. cerebriformis* in skin. Hematoxylin and eosin stain. × 1,220.



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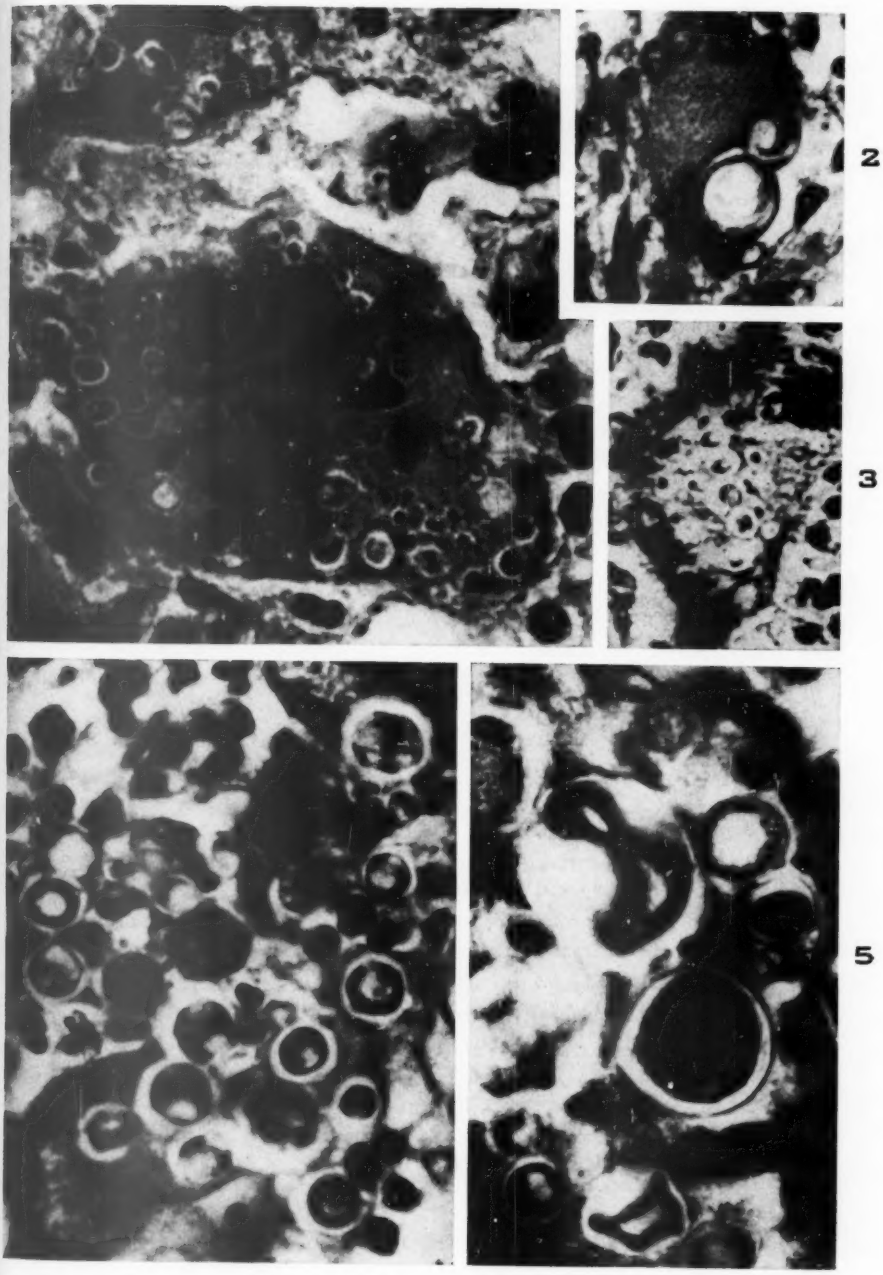
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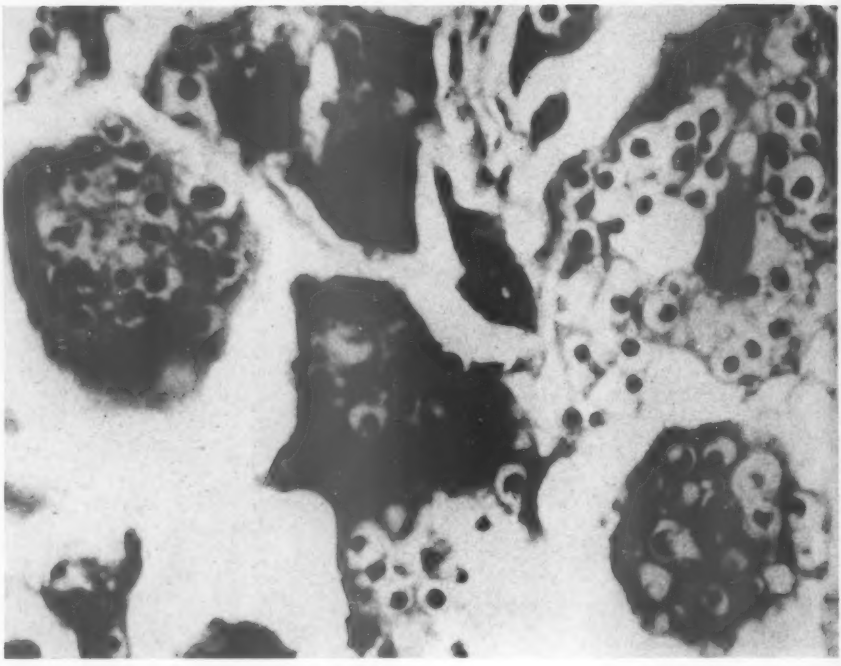
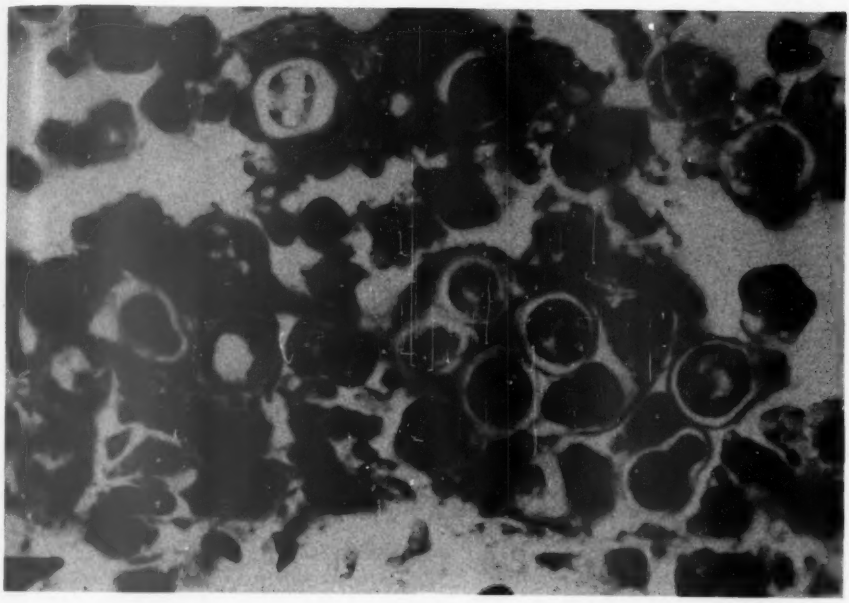


Fig. 6. Small forms of *Histoplasma capsulatum* within histiocytes. Crescent-shaped chromatin may be noted within cells. Hematoxylin and eosin stain. × 1,730.

Fig. 7. Simple and budding large forms of *H. capsulatum* in prostatic tissue. Hematoxylin and eosin stain. × 1,540.

Fig. 8. Budding and germinating cells of *Cryptococcus neoformans* in section of liver. Periodic acid-Schiff's stain. × 940.

Fig. 9. Varied forms of *H. capsulatum* in vegetation from aortic valve. Giemsa's stain. × 890.



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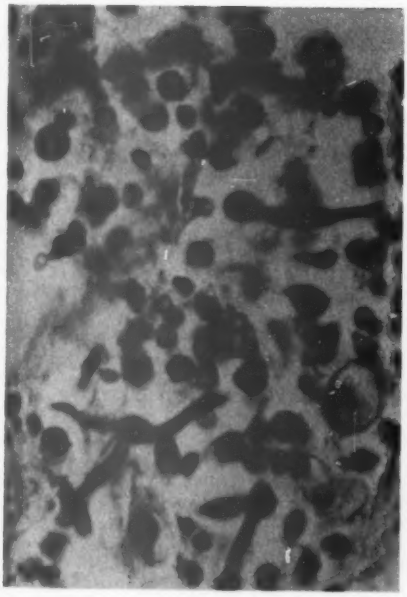

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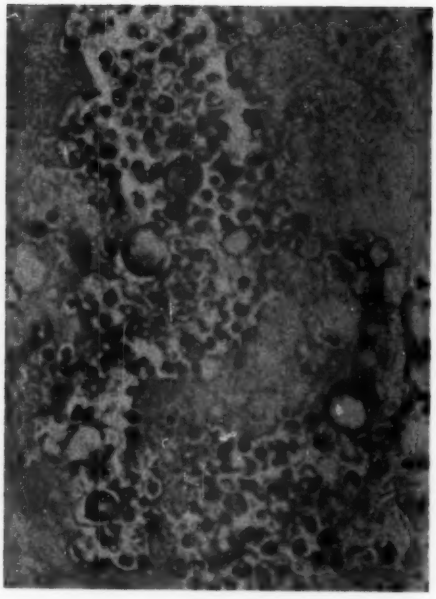

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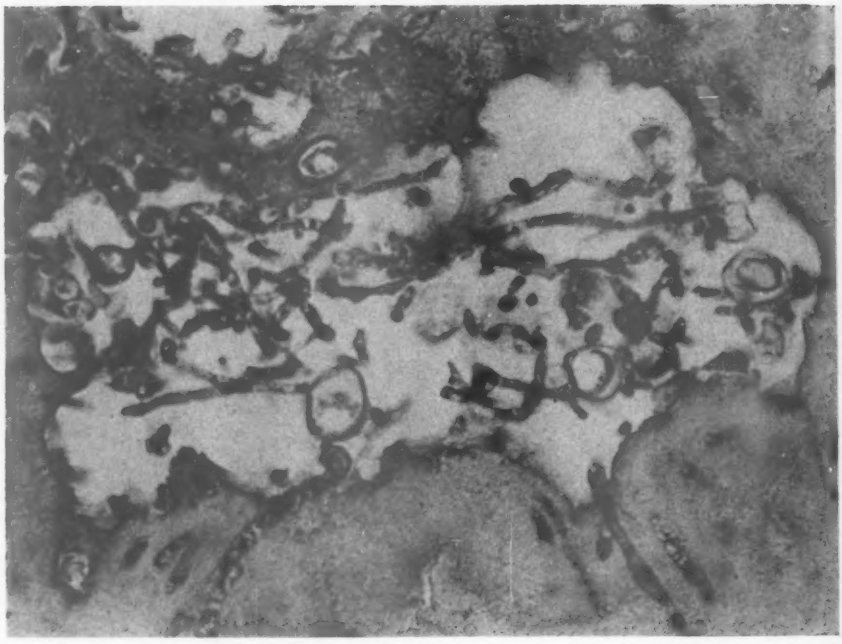

FIG. 10. Filaments and small and large cells of *H. capsulatum* in a space (probably fluid) within vegetation on aortic valve. Filaments extend into the granulation tissue. Giemsa's stain. × 820.

FIG. 11. Multiple budding forms of *P. brasiliensis* in tissue. Small buds may be noted. Hematoxylin and eosin stain. × 1,220.

FIG. 12. Multiple budding form of *P. cerebriformis*. Large buds may be seen. Hematoxylin and eosin stain. × 1,220.

FIG. 13. Multiple and simple budding forms of *P. brasiliensis* in tissue. Hematoxylin and eosin stain. × 1,930.

FIG. 14. Multiple budding forms of *Saccharomyces cerevisiae* grown in medium containing 1:10,000 dilution of sodium silicofluoride. Unstained wet mount. × 970.

FIG. 15. Multiple budding forms of *Sacch. cerevisiae* grown in medium containing 1:1,000 dilution of sodium fluoride. Unstained wet mount. × 970.

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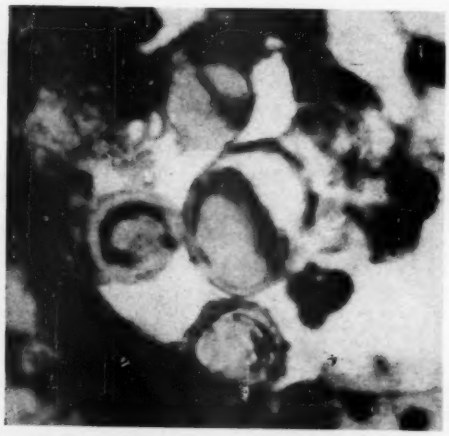

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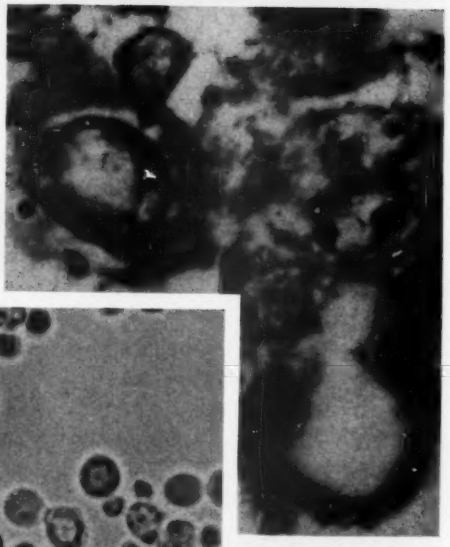

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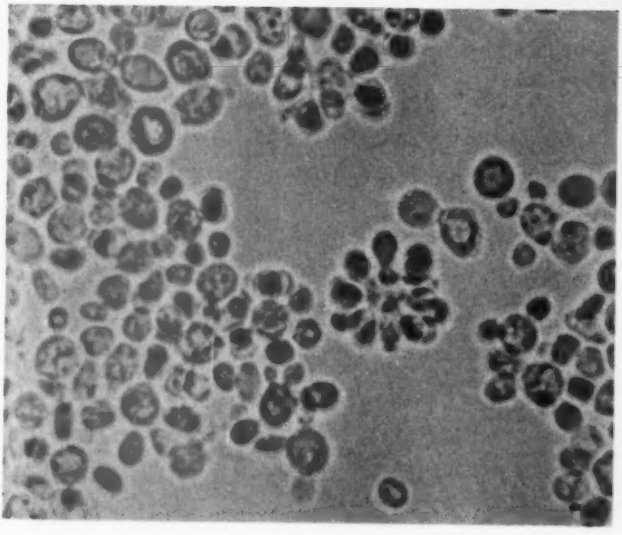

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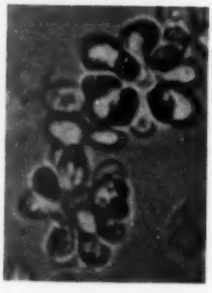

15

IMMUNIZATION AGAINST BRUCELLA INFECTION

V. Histopathologic Appraisal of Immunity Induced in Mice by a Streptomycin-Dependent Mutant of Brucella melitensis *

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Earlier publications have dealt with active immunization against experimental infection by *Brucella melitensis* in mice, guinea-pigs, and monkeys, using as an immunizing agent a living attenuated strain of *B. melitensis*.[1-4] The vaccine strain was characterized by a nutritional dependence upon streptomycin and was in consequence capable of minimal multiplication in the injected host. By virtue of its invasiveness, multiplication, and temporary persistence, the drug-dependent mutant produces a powerful immunogenic effect. As living complete antigenic units, the cells of the vaccine suspension are brought into more intimate contact with the reticulo-endothelial cells, lymphocytes, etc., and leave, as in tuberculosis, permanent imprints on these cells and their descendants.

The streptomycin-dependent mutant offered the opportunity for the first time of studying an immune process active against *B. melitensis* in a reproducible manner. It was therefore the objective of the present study to supplement the bacteriologic data with an intensive appraisal of the histopathologic aspects of the immune response. It is recognized, however, that in brucella infections, as in tuberculosis, such reactions are accompanied and complicated by an intercurrent allergic inflammatory reaction. Thus, the unravelling of the immune and allergic responses remains an experimental challenge to subsequent investigation of this subject. In the light of the development of a reagent for the detection of the allergic state in a degree of purity not heretofore available,[5,6] a powerful tool is available to begin the investigation of the interconnections between the allergic and immune state. This will be the subject of a later communication.

Materials and Methods

Experimental Animals. Adult male mice of the Webster BRVS strain, weighing 18 to 20 gm., were used.

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Immunization. Five billion cells of the streptomycin-dependent strain of *B. melitensis*[1] were injected twice subcutaneously at an interval of 3 weeks. After a further resting period of 4 weeks the immunity was tested by injection of a virulent strain of *B. melitensis.*

Infection. The basic portion of this study involved the comparative responses of normal and immunized mice to an injection of 5×10^9 cells of a virulent *B. melitensis* strain, M6056. In addition, groups of normal and immunized mice were studied for their responses to the relatively avirulent vaccine strain in both the living and heat-killed state and finally to heat-killed cells of the virulent strain.

Thus, 8 groups of animals were available for the study as follows:

Group 1. Normal animals *vs.* viable vaccine strain cells.
Group 2. Normal animals *vs.* heat-killed vaccine strain cells.
Group 3. Normal animals *vs.* viable virulent strain cells.
Group 4. Normal animals *vs.* heat-killed virulent strain cells.
Group 5. Immune animals *vs.* viable vaccine strain cells.
Group 6. Immune animals *vs.* heat-killed vaccine strain cells.
Group 7. Immune animals *vs.* viable virulent strain cells.
Group 8. Immune animals *vs.* heat-killed virulent strain cells.

Each group was comprised of 50 mice. Mice were sacrificed from each group at 1, 3, 6, and 24 hours, 2, 3, 4, 5, 7, 14, 21, 28, 35, and 42 days.

Histologic Methods. Specimens of skin, including the site of injection, regional lymph node, spleen, and liver were fixed in Bouin's solution for 24 hours. Without washing, the tissues were dehydrated and embedded according to the basic nitrocellulose method of Koneff and Lyons.[7] In view of the lack of sufficient standardization and unanimity of nomenclature among the various producing companies, one type of nitrocellulose was used throughout this work. It was obtained from the Hercules Powder Company, Parlin, N.J., and designated as "Type RS 1/2 sec. N/C; Viscosity 3-20 sec."

Sections were cut 4 μ thick on the sliding microtome, mounted with albumin, and immersed immediately in clove oil which both fixed the albumin and dissolved the impregnated nitrocellulose. The slides were left at least 24 hours in clove oil to insure complete solution of nitrocellulose.

Clove oil was removed by four washings in absolute ethyl alcohol after which the sections were rehydrated.

It was found advisable at this point to treat the tissues for 2 hours in a 2.5 per cent solution of $K_2Cr_2O_7$. Experience showed that when working with fixed tissues, exposure to $K_2Cr_2O_7$ greatly enhanced the eosinophilic qualities when using the Giemsa stain.

The slides were washed in distilled water for about 5 minutes, with constant agitation. The tissues were then stained by a modification of the Gradwohl-Giemsa method for thick blood smears and further differentiated according to the procedure of Krajian and Gradwohl.[8] The sections were mounted in piccolyte.

Bacteriologic Methods. A portion of each spleen, regional lymph node, and skin at the site of injection was ground in sterile sand, suspended in sterile saline solution, and plated in duplicate on either plain Albimi agar (for the virulent 6056 strain) or on Albimi agar to which was added 500 μg. of streptomycin per ml. to support growth of the drug-dependent strain, SMd. In addition to specimens of these organs, a sample of the heart's blood was taken from each animal and cultured.

Since the virulent 6056 strain grew more rapidly than the SMd strain, plates containing the former organisms were examined after 48 hours of incubation. The plates were examined under the 3 × magnification of a dissecting microscope, and positive plate cultures were recorded at this time. The negative plates were held for an additional 5 days of incubation and re-examined. If there was no evident growth at the end of a week, the specimen was recorded as negative.

For the SMd strain, the first examination of the plates was made after 7 days of incubation. Negative plates were held in the incubator for another week, re-examined, and if no growth was evinced, they were recorded as negative.

Results were recorded as positive if even a single colony on either of the two plates, for either animal at the given time level, was obtained.

Results

Bacteriologic Observations

Of the eight groups involved in the experiment, only four warrant bacteriologic observation because only four groups received viable organisms.

Table I gives a resumé of the clearing process beginning at 24 hours. It shows that there is a distinct difference between the time of clearance for group I and group V. When the normal animal was infected with SMd, the first signs of bacteriologic clearance were evinced at 2 weeks. But when the immunized animal was challenged with this same organism (a strictly homologous system), clearance already had begun to take place on the first day, and by the end of a week the animal had rid itself of the organism.

Group III gives the typical picture of brucella infection in a normal

mouse. The bacteremia disappeared within 1 week, but the organisms lodged and continued to grow in the skin and other tissues of the body. They disappeared from the liver and skin by the fifth week, and the infection remained only in the spleen and lymph node. A few mice were held over from this group and examined after 10 weeks (not recorded in Table I). By that time the lymph nodes were clear, but organisms were still present in the spleen.

TABLE I

Comparison of Plate Counts

	1 day	2 days	3 days	4 days	5 days	7 days	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.
Group I SMd *vs.* normal											
Skin	+	+	+	+	+	+	±	+	±	−	−
Spleen	+	+	+	+	+	+	+	−	−	−	−
Liver	+	+	+	+	+	+	+	−	−	−	−
Heart blood	+	+	+	+	+	+	+	−	−	−	−
Lymph node	+	+	+	+	+	+	±	+	±	−	−
Group V SMd *vs.* immune											
Skin	+	+	+	±	−	±	−	−	−	−	−
Spleen	±	−	±*	−	−	−	−	−	−	−	−
Liver	−	−	−	−	−	−	−	−	−	−	−
Heart blood	±	±	±	±*	−	−	−	−	−	−	−
Lymph node	0	+	+	+	+	−	−	−	−	−	−
Group III 6056 *vs.* normal											
Skin	+	+	+	+	+	±	+	+	+	−	−
Spleen	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	−	−
Heart blood	+	+	+	+	+	−	−	−	−	−	−
Lymph node	+	+	+	+	+	+	+	+	+	+	+
Group VII 6056 *vs.* immune											
Skin	+	−	−	−	−	−	−	−	−	−	−
Spleen	+	+	+	+	−	−	−	−	−	−	−
Liver	+	+	+	±	−	±	−	−	−	−	−
Heart blood	+	+	±*	−	−	±	−	−	−	−	−
Lymph node	+	+	±	±	−	−	−	−	−	−	−

± Means one plate was positive, the other negative.

* Plate of the one animal that was positive had only two colonies.

A comparison of group VII with group III shows the protection afforded by the vaccine. In the immune group VII, bacterial clearance was completed at the site of injection within 2 days; the spleen cleared in 4 days, the liver in 3 to 4 days, the heart's blood in 3 days, and the lymph node in 3 to 4 days. No signs of infection were observed after 1 week. These results were checked by inoculating another series of 35 immune animals and using the tissues solely for bacteriologic

study. The results were qualitatively the same, as is shown in Table I.

A comparison of group V with group VII shows that the relatively avirulent strain was more rapidly cleared than the virulent strain.

MORPHOLOGIC OBSERVATIONS

Response of Normal Mice to Injection of Living Virulent B. melitensis (Groups III and IV)

Injection Site. As the reaction in groups III and IV was followed over the 6 weeks' period, the site of injection showed a prompt phagocytic (chiefly microphagic) response to the organisms. The reaction was inadequate, so that bacterial multiplication was allowed and visible organisms persisted at the site for the full period of study. In fact, the microphages themselves underwent necrosis and their bacteria seemed to grow as new colonies. The lesion was eventually circumscribed by granulation tissue and in some cases sloughed to the surface. A moderate plasma cell reaction was demonstrable at the margins.

Lymph Nodes. Except for changes in some nodes caused by direct contact with the injected site, the chief lesion in the lymph nodes was the development of small epithelioid nodules. These were first recognized *at 2 days* as small groups of large mononuclear and polymorphonuclear leukocytes. They were found both in the sinusoids and pulp. By 5 days an epithelioid character was more pronounced. Bacteria were not seen in them at any stage. At 6 weeks the lesions were resorbing. The nodes showed also a gradual increase in plasma cells.

Liver. The livers in this series exhibited the gradual development of many small epithelioid nodular lesions in the sinusoids, seen best at 5 and 7 days. At 24 hours there was a diffuse Kupffer cell hyperplasia. Apparent resorption of the lesions was occurring by the sixth week.

Spleen. Excessive numbers of polymorphonuclear leukocytes and sinusoidal macrophages were present in the spleen after 24 hours. By 5 and 7 days, many small, distinct, epithelioid, nodular lesions, resembling those in the liver, were present, persisting to the sixth week, although by that time they appeared to have regressed. The plasma cell reaction was not conspicuous. At the height of the epithelioid nodular reaction, the lymphoid tissue was atrophic.

When the same number of heat-killed cells of this virulent strain were injected, the bacteria were phagocytosed in 24 hours and disappeared from the site of injection. There was no evidence of dissemination to the regional lymph nodes, spleen, or liver. At the site of injec-

tion, in marked contrast to the observations with viable virulent cells, the host cells did not undergo massive necrosis and there was no ulceration of the surface. The local lesion was practically healed by 5 to 6 weeks.

The Response of Immune Mice to Injections of Virulent B. melitensis (Groups VII and VIII)

Injection Site. At 1 hour, the large bacterial mass in the injection site of groups VII and VIII showed a few scattered, bacteria-laden phagocytes, chiefly microphages. In 3 hours an intense marginal zone of polymorphonuclear leukocytes gathered around the bacterial mass, but evidenced little penetration. Some of these cells were phagocytic. At 6 hours, the leukocytic zone was more intense and its outer part contained many eosinophils; bacteria were spreading outside this cellular zone from the very large central colony. At 2 days, no free bacteria were seen. The bacterial mass was infiltrated almost solidly by microphages. Many eosinophils were found at the margins of the lesion. At 4 days, many of the large, central microphages appeared to be degenerating and fine, granular bodies were seen in the central area. At 5 days, there was more central degeneration of leukocytes. One area showed liquefactive necrosis and large, irregular, blue-black masses. There was some fibrin at the margin of the lesion. The surrounding tissues showed an intense, large, mononuclear cell infiltration, in which there were some plasma cells. At 1 week, the lesion resembled the 5-day stage, but many macrophages were present at the margin of the polymorphonuclear cells and there were fibroblasts external to them.

Lymph Nodes. No nodular epithelioid lesions were seen in the lymph nodes at any stage. The nodes showed a mild lymphadenitis during the first week, characterized by excess numbers of polymorphonuclear and monocytic leukocytes in the sinusoids. In the second week, some of the sinusoidal macrophages were clumping.

Liver. This series showed the same perivascular small round cell foci in the liver as the others. In addition, at 4 and 5 days and at 3 weeks there were scattered small sinusoidal nodular lesions. In the first week they consisted of large round cells and polymorphonuclear cells, but at 3 weeks they had an epithelioid appearance. However, similar lesions were seen at 1 hour, in all probability a result of the immunization procedure with the drug-dependent mutant.

Spleen. No specific brucella lesions were seen in the spleen except, possibly, one epithelioid nodule at 3 weeks. Some of the sections

showed excessive numbers of polymorphonuclear leukocytes in the sinusoids. Plasma cells were conspicuous in most of the series.

In summary, this series showed prompt localization and control of the challenge strain of bacteria at the injection site, and minimal evidence of dissemination to the regional lymph nodes, spleen, and liver. The local reaction was an intense polymorphonuclear cell infiltration with engulfment of all bacteria by 24 hours. This lesion had considerable numbers of eosinophils. A large lesion was produced, which did not excavate to the surface in the sections examined. There was some suggestion of, but no definite evidence for, dissemination from the injection site.

The response of this group of previously immunized animals to injection of heat-killed cells of strain 6056 was quite similar qualitatively and quantitatively. Prompt mobilization of neutrophilic and eosinophilic polymorphonuclear leukocytes and perhaps more rapid accumulation of large mononuclear cells characterized this response, which also included plasma cells at the margin of the lesion. Minimal spread to the regional lymph nodes occurred but no dissemination to the liver and spleen.

Response of Mice to Injection of the Streptomycin-Dependent (SMd) Mutant Strain of B. melitensis (Groups I and II)

Injection Site. At 1 hour, the injection site of groups I and II showed a little fluid containing scattered polymorphonuclear leukocytes. At 6 hours, the injection site was moderate in size, having a small amount of fluid and containing large numbers of bacteria, diffusely scattered and in colonies. There was a great polymorphonuclear leukocytic reaction to the bacteria, principally diffuse, but focal and intense in a few places. A few monocytes were present. Both macrophages and microphages were evident; the latter were still quite small and had central nuclei. At 24 hours, the lesion was larger, and the bacterial colonies were densely infiltrated by polymorphonuclear leukocytes, many of which were greatly enlarged, and appeared as bacteria-stuffed microphages. A few large macrophages were seen also. All bacteria were intracellular. At 2 days, the injection site was not circumscribed. Many extracellular bacteria were present, and great numbers of large microphages, hardly recognized as such because of the intensity of the phagocytosis, were present in these areas. A few large macrophages were present also. At 3 days, large numbers of extracellular and intracellular bacteria were seen, the latter chiefly in microphages. The leukocytic response to the bacteria was dense and

intense, and some areas showed degeneration of leukocytes (suppuration). There was a little marginal deposition of fibrin. Many of the scattered peripheral leukocytes were slightly eosinophilic. At 4 days, the lesion was an early abscess, with a distinct fibrinopurulent membrane in which microphages, appearing to contain bacteria, were conspicuous. At 5 days, the lesion comprised an intense focus of polymorphonuclear cells, some of which showed karyorrhexis while others were enlarged and had peripherally arranged nuclei; no bacteria were visible. Large macrophages with abundant blue cytoplasm were found in and at the margins of the main lesion. At 1 week, a large abscess was seen, with partial necrosis of the overlying skin. Its center was packed with microphages, apparently laden with bacteria. At the deep margin, in the muscle, was a zone of mononuclear cells and fibroblasts, containing a few eosinophils. There was some plasma cell reaction in the surrounding tissues. At 2 weeks, the lesion was an abscess which was draining through a sinus but it still contained large numbers of microphages and large blue-staining macrophages. A marginal fibroblastic layer was quite distinct. At 3 weeks, the large abscess, containing many microphages and macrophages, had at its margin a zone of fibroblastic and mononuclear cells which was wide and poorly delimited, and contained a few groups of plasma cells. Mast cells appeared in greater than normal numbers. At 6 weeks, the section showed the remnants of a drained, collapsed lesion consisting chiefly of fibroblasts, large mononuclear cells, and a few plasma cells.

In summary, the sequence was that of a large, acute, inflammatory reaction, progressing to abscess formation, fibrous encapsulation and, in several animals, to sinus formation to the exterior and partial resorption in 6 weeks.

Regional Lymph Nodes. The picture in the regional lymph nodes in response to the dependent mutant strain was that of an acute lymphadenitis, visible at 1 hour, maximal about the fifth day, which subsided almost completely by the sixth week when the node appeared relatively normal. It was characterized by mononuclear cell hyperplasia which chiefly involved the sinusoids, but was seen also in the cords. At the height of the reaction, some diffuse hyperplasia of reticulum cells was seen also in the lymphoid nodules, together with some atrophy of the lymphoid cells. Bacteria were not identified in the lymph nodes, and no nodular lesions were seen resembling those in the liver and spleen. Great numbers of plasma cells were seen, and these appeared to have undergone a gradual increase.

Liver. This series of sections showed the development and eventual virtual disappearance of small, microscopic nodular lesions in the hepatic sinusoids, composed chiefly of large mononuclear macrophages. A slight, diffuse hyperplasia of the Kupffer cells was apparent at the same time. The largest lesions extended beyond single sinusoids, and showed concentric layers of epithelioid cells. Bacteria were not identified at any period. The liver cells showed minor retrogressive changes, interpreted as toxic, at the height of the Kupffer cell reaction.

The first change was seen at 24 hours, and was characterized by enlargement and apparent increase in sinusoidal cells, in some places forming small groups of cells. Up to 3 weeks, such focal lesions increased in size and numbers. At 5 and 6 weeks, regression was almost complete.

Spleen. In general, the sequence of changes in the spleen resembled that described in the liver, in addition to which a great increase was seen in polymorphonuclear leukocytes. The size of the spleen did not seem to change very much. At 1 hour many polymorphonuclear leukocytes were present and at 2 days a considerable hyperplasia of sinusoidal mononuclear cells was found, as well as great numbers of polymorphonuclear cells. Innumerable miniature nodular epithelioid-cell lesions were present at 2 and 3 weeks, but at 6 weeks they had virtually disappeared, leaving no residues. Bacteria were not recognized at any time.

The response to the living dependent mutant cell was in general characterized by a great multiplication of bacteria at the injection site despite considerable phagocytosis by microphages and macrophages, and considerable delay in disposal of the bacteria. There was local abscess formation with self-drainage. The regional lymph nodes showed mild local extension of the infection and the liver and spleen showed evidence of septicemia. Small epithelioid granulomas were formed in them which, by 6 weeks, virtually disappeared.

In those animals receiving heat-killed cells of this strain, the injection site was brought under control within 6 weeks. There was dissemination to the regional lymph nodes but questionable hematogenous dissemination of these organisms to the liver and spleen. A conspicuous plasma cell reaction was seen in the lymph nodes and spleen. The cellular reaction to the heat-killed SMd cells was prompt but not great and penetration into the bacterial colony with phagocytosis and removal was slow.

Response of Vaccinated Mice to Reinjection of the Living Vaccine Strain (Groups V and VI)

There was a large and prompt reaction to the bacteria at the injection site of groups V and VI and no evidence for the dissemination of the bacteria to the regional lymph nodes, liver, or spleen. The bacteria were disposed of promptly by microphages and macrophages. The promptness and intensity of the local reaction with a high component of eosinophils, its failure to liquefy, its tendency to undergo coagulation necrosis, the presence of many plasma cells at the periphery of the lesion, and the successful restriction of the bacteria to the injection site, characterized this as an immune reaction.

The response of this class of mice to injections of heat-killed cells of strain SMd can be described in the same terms as in the preceding paragraph. The lesion was smaller and was resorbed more quickly.

Summary and Conclusions

Immunized animals reacted to virulent *Brucella melitensis* in a manner quantitatively as well as qualitatively different from normal animals. The immune mice showed a more intense leukocytic response, in them penetration of the bacterial colony was more prompt, and a more rapid clearance of the microorganisms was found. These were accompanied also by less degeneration of the microphages themselves in the process. Furthermore, in immune animals no proof of dissemination of the organisms from the injection site was found, in marked contrast to that seen in unvaccinated animals.

Immunized animals reinfected with the attenuated vaccine strain showed at the injection site evidences of anaphylactic inflammation. Invasion and disposal of the bacterial mass were accelerated markedly in vaccinated animals, which also exhibited more coagulative and less liquefactive necrosis than was seen in animals which had not had prior contact with the strain. Again, in the immunized series no specific brucella lesions developed in the regional lymph nodes, spleen, and liver resembling those seen in animals injected for the first time with the vaccine strain.

Heat-killed cells of the virulent strain of *B. melitensis* incited a more efficient inflammatory response than did heat-killed cells of the attenuated vaccine strain. The leukocytes permeated the lesion caused by the virulent strain in 24 hours as contrasted to 72 hours in the lesion caused by the vaccine strain. On the other hand, the vaccine strain incited an extensive plasma cell reaction at the injection site as well as in the regional lymph nodes, which began on the third post-injection

day and persisted for 4 weeks. This was not observed in the animals receiving heat-killed cells of the virulent strain.

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CANCER OF THE CERVIX UTERI

A Review of 169 Necropsied Cases *

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It has been demonstrated[1] that radical hysterectomy with bilateral pelvic lymph node dissection can be performed with a very low mortality, small morbidity, and satisfactory results in patients with stage I and stage II carcinoma of the cervix. Recently, attempts have been undertaken to achieve surgical results better than those derived from radiation therapy in stages III and IV. It has been established that radical pelvic exenteration can be accomplished with a mortality of 25 per cent or less and that the resultant physiologic readjustment permits a fairly comfortable and active life.[2,3] Radical removal of all pelvic viscera for advanced cervical cancer apparently should be limited to patients in whom a chance for cure by any other form of therapy is less than 5 per cent.

For a better understanding of the pathologic features and the actual extent of the disease, we believed that a complete review of the necropsied cases of carcinoma of the cervix which had been treated by radiation would be of great value. From this we hoped to make a better evaluation of the possibilities of ultra-radical surgery. The findings at necropsy should also give us a picture of the life history of cancer of the cervix and perhaps an idea of the value of, and the complications from, radiation therapy.

This report represents the study of 169 cases of carcinoma of the cervix treated by radiation and necropsied at the Pondville Cancer Hospital from 1931 to 1951.

Types and Histologic Grading

From our study it is evident that the significance of histologic grading of neoplasms is far from settled, and is of less importance than the clinical extent of the disease. In determining the histologic grading, a modification of Broders' classification proposed by Warren[4] was used.

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It divides the epidermoid carcinomas into three groups: grade I, low malignancy; grade II, medium malignancy; and grade III, high malignancy. The histologic criteria are based upon the degree of differentiation of tumor cells, relation of tumor to stroma, and frequency of mitotic figures; the last being considered the most important single

TABLE I

Histopathologic Grading of all Epidermoid Tumors

Grade I		Grade II		Grade III		Total	
No.	%	*No.*	%	*No.*	%	*No.*	%
17	11.5	68	46.2	62	42.1	147	100

TABLE II

Type of Tumor

Epidermoid		Adenocarcinoma		Adeno-acanthoma		Total	
No.	%	*No.*	%	*No.*	%	*No.*	%
147	87.0	13	7.7	9	5.3	169	100

criterion. The average number of mitotic figures per high-power field was established for the purpose largely of eliminating the personal equation. It was considered that an average of less than two mitotic figures per high-power field constituted grade I; from two to four, grade II; and more than four, grade III. The adeno-acanthomas and adenocarcinomas were considered in a separate classification.

In all of the necropsied cases, except two in which no tumor was found, the histologic grading was checked from necropsy material. For the two cases, we used the biopsy slide.

A slightly greater incidence of distant metastases was noted in grade III carcinomas and in the adeno-acanthomas, but no significant difference was noted as to pelvic extension among the grades and types.

Frequently, at necropsy, the pelvic extension of the process could not be determined accurately because of marked fibrosis due to radiation. This change may be difficult to differentiate grossly from neoplastic involvement.

Incidence and Distribution of Distant Metastases

It is well known that carcinoma of the cervix exhibits a low incidence of distant metastases and more often spreads locally into the parametrium, pelvic organs, and pelvic lymph nodes. Lack of spread may be explained partly by the frequent occurrence of early ureteral obstruction with resultant uremia and death and therefore lack of time

for further involvement. In our series, 22.4 per cent of the 169 necropsied cases showed evidence of distant metastases. This figure is slightly lower than those of other reports. The great majority of patients dying of cervical cancer do not have metastases outside the pelvis. In every instance in which there was metastasis to the nodes at the bifurcation of the aorta, distant metastases were found also.

TABLE III

Incidence and Anatomical Distribution of Distant Metastases in Cases of Carcinoma of the Cervix Uteri in Which the Periaortic Lymph Nodes Were Involved

Liver	41 (85.4%)
Lungs	31 (64.5%)
Peritoneum	24 (50.0%)
Bone	14 (29.3%)
Miscellaneous	33 (68.7%)

(22.4% of all cases in the necropsied series exhibited distant metastases.)

TABLE IV

Anatomical Distribution of the 33 Miscellaneous Distant Metastases

Kidney	7
Adrenal gland	7
Intestine	6
Spleen	5
Heart	3
Brain	2
Pancreas	2
Skin	1

TABLE V

Anatomical Distribution of Osseous Metastases

Lumbar vertebrae	14
Dorsal vertebrae	4
Humerus	2
Ribs	1
Skull	1
Femur	1

(8.2% of all cases showed bony metastases; more than one bone was involved in some patients.)

TABLE VI

Incidence of Ureteral Obstruction

Left	18 (15.0%)
Right	16 (13.3%)
Bilateral	86 (71.6%)
Total	120

(71% of the 169 cases showed either bilateral or unilateral ureteral obstruction.)

Distant metastases were not encountered in this series in the absence of periaortic nodal involvement. This observation seems to suggest that the lymphatic system is the usual route through which cervical cancer spreads to distant regions, and the rarity of blood vessel invasion in microscopic sections is supporting evidence. In cases with distant metastases, the lungs were involved in 64.5 per cent and the bones in 29.3 per cent.

Incidence of Ureteral Obstruction

The ureteral and renal complications of carcinoma of the cervix have been recognized since the middle of the last century. In 1858, Wagner[5] reported that one third of all his cases showed ureteral involvement on examination at necropsy. Williams,[6] in 1895, reported

an incidence of hydro-ureter and hydronephrosis of 85.9 per cent in 78 necropsies. In the 150 post-mortem examinations of Faerber,[7] obstructive renal changes were encountered in 72 per cent. Behney,[8] in 166 cases, reported the incidence of ureteral obstruction as 65 per cent, and the occlusion was sufficient to cause uremia and death in 21 per cent of patients. Warren[9] ascribed the great majority of deaths in cervical cancer to renal insufficiency of obstructive nature.

Graves, Kickham, and Nathanson,[10] in a very comprehensive analysis of 600 cases of carcinoma of the cervix at the Pondville Cancer Hospital from 1927 to 1935, with 87 necropsies, some of which are included also in the present communication, found ureteral obstruction in over 70 per cent. In this present series 71 per cent of the 169 necropsied patients showed either bilateral or unilateral ureteral obstruction. The obstruction was severe enough to cause uremia and death in 46.1 per cent.

Causes of Death

Our analysis showed that uremia was by far the most frequent cause of death. The second most frequent cause of death was peritonitis.

TABLE VII

Causes of Death in Carcinoma of the Cervix

Uremia	78 (46.1%)
Peritonitis	38 (22.4%)
Pneumonia	11 (6.5%)
Hemorrhage	5 (2.8%)
Intestinal obstruction	3 (1.7%)
Miscellaneous*	35 (20.7%)
Total	169

* In 80% of the miscellaneous group of 35 it was believed that death was due to cachexia and/or carcinomatosis.

TABLE VIII

Incidence of Fistula Formation According to Anatomical Location

Vesico-vaginal	52 (58.4%)
Recto-vaginal	34 (38.2%)
Miscellaneous	3 (3.3%)
Total	89 (100.0%)

Necropsies showed that 22.4 per cent of the cases had peritonitis resulting from post-radiation necrosis and perforation. One of the two cases in which no tumor was found at necropsy had severe radiation fibrosis with necrosis of the bowel and perforation and peritonitis.

In 35 cases, or 20.7 per cent, the immediate cause of death could not be determined accurately; in 80 per cent of these 35 cases death was attributed to cachexia and/or carcinomatosis. Pneumonia accounted for 6.5 per cent of all deaths. Hemorrhage was responsible for only 2.8 per cent, and in all cases was due to tumor invasion and necrosis of the iliac veins. The arteries appeared to be extremely resistant to neoplastic involvement.

Incidence of Fistulas

All cases in this series were treated with radium, so it is not possible to compare the incidence of fistulas with that in a group of untreated cases. Warren[4] found no significant difference in such groups, namely, 43 per cent of those who had radium treatment and 46 per cent of those with no treatment. At necropsy 67 of our 169 cases showed one fistula, or an incidence of 40 per cent, and 22 of them exhibited two fistulas. The total number with fistulas was 89, or 52 per cent. In many of the cases the tissues surrounding the fistula showed changes which strongly suggested radiation change as the primary cause of the lesion.

Summary and Conclusions

In cervical cancer the disease is limited to the pelvis in the majority of the cases, so that theoretically a complete removal of pelvic viscera should be curative.

When a pelvic exenteration is contemplated, a complete study of the patient should be carried out. Roentgenograms of the chest and a radiologic bone survey should be made, and tests of renal function should be performed. Only by a complete examination can unnecessary surgery be avoided.

Accepting the fact demonstrated by this study that metastasis to the lymph nodes at the bifurcation of the aorta is invariably accompanied by distant spread, we recommend that before proceeding with exenteration these nodes should be subjected to pathologic examination. Frozen sections are adequate for this purpose in the hands of a competent surgical pathologist.

Ureteral obstruction was found in 71.0 per cent of this group of necropsied patients. Often the ureteral obstruction was bilateral.

Fistulas are frequent, due either to the progress of the disease or to severe radiation change.

The most frequent causes of death are uremia and peritonitis.

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RELAPSING FEVER IN KOREA

A Clinicopathologic Study of Eleven Fatal Cases with Special Attention to Association with Salmonella Infections *

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Epidemics of louse-borne relapsing fever have been reported by many observers in countries where the disease was endemic and where facilities for sanitation were disrupted because of war or civil strife.[1-5] Such circumstances existed in Korea during 1950 and 1951. Relapsing fever was known to be endemic in Korea,[6] and during the early active phases of the war in Korea in 1950 and 1951, facilities for sanitation in prisoner-of-war camps often proved to be inadequate because of overcrowding and failure to observe sanitary discipline. Experiences during World War II with relapsing fever among Chinese troops who were flown into Burma indicated that reservoirs among migrant Chinese troops in Korea were another probable source of the disease.[7]

From August, 1950, to December, 1951, cases of relapsing fever were recognized among United Nations personnel and prisoners of war in Korea (Table I).[8] In the majority of cases the diagnosis was established readily by demonstration of spirochetes in blood smears. However, complications due to coexisting Salmonella and dysentery infections, hepatitis, and hemorrhages of the central nervous system frequently confused clinical and post-mortem interpretation of fatal cases (Table II).

Clinical and laboratory observations and results of therapy have been recorded in considerable detail in previous epidemics of louse-borne relapsing fever,[4,5,7] but the pathologic changes in fatal cases seldom have been described. The purpose of this paper is to record the clinical and pathologic data in 11 proved necropsy cases, but particular emphasis will be placed on those complicated by Salmonella or Shigella infection.

The first case was one of spontaneous rupture of the spleen in an American soldier, previously reported by Legerton and Chambers[9] in 1950. The 10 more recent cases occurred among United Nations per-

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sonnel and prisoners of war in Korea during 1950 and 1951. Relapsing fever was not suspected by clinicians or pathologists in several early cases. The first fatal case of 1951 (case 2) was one in which clinical symptoms and signs were referable to hemorrhage in the central nervous system. At post-mortem examination, unfamiliar follicular changes were observed in the spleen as well as subdural and intracerebral hemorrhages, but the etiology of these lesions remained obscure until several months later when their relationship to relapsing fever was recognized. Tissues from this case were stained by the Warthin-Starry method, and spirochetes were found in the brain, spleen, and all other viscera examined.

TABLE I

*Reported Cases of Relapsing Fever in Prisoners of War and in United Nations Forces in Korea During 1951**

1951	U. S. Army	Prisoners	Total
January	2	0	2
February	0	0	0
March	0	30	30
April	1	10	11
May	14	10	24
June	19	36	55
July	13	12	25
August	2	2	4
September	0	4	4
October	4	1	5
November	4	2	6
December	1	0	1
Totals	60	107	167

* There were 8 additional cases reported during 1950; all were in prisoners and during the month of August.

Subsequently, the association of Salmonella septicemia and relapsing fever was observed.[10] Two patients (cases 10 and 11) with a clinical diagnosis of relapsing fever (Table III), established by demonstration of Borrelia in blood smears, were treated with mapharsen. Both patients died. At post-mortem examination a diagnosis of Salmonella septicemia was established in both cases by cultures of blood and tissues. Silver stains performed on all tissues were negative for spirochetes.

After the association of Salmonella septicemia and dysentery with relapsing fever became apparent, all necropsy cases with an established diagnosis of Salmonella septicemia, shigellosis, or splenomegaly of undetermined origin were reviewed and appropriate tissues were stained for spirochetes. In a group of 20 such cases, 4 additional cases of relapsing fever were discovered.

On the basis of clinical and pathologic findings, the cases were divided into the following groups:

I. Relapsing fever uncomplicated by Salmonella septicemia or shigellosis (cases 1 to 5).

II. Relapsing fever with coexisting Salmonella or Shigella infection (cases 6 to 9).

III. Relapsing fever treated and cured, with death due to Salmonella septicemia (cases 10 and 11).

The means by which a diagnosis of relapsing fever was established

TABLE II

Diagnoses Made in 11 Cases of Relapsing Fever

	CASE NUMBER	ADMISSION DIAGNOSIS	FINAL CLINICAL DIAGNOSIS	GROSS AUTOPSY DIAGNOSIS	FINAL AUTOPSY DIAGNOSIS
I	1. J-563	1-Prodrome of Infectious Hepatitis.	1-Ruptured Spleen of Undetermined Cause.	1-Ruptured Spleen Due to Relapsing Fever.	1-Ruptured Spleen Due to Relapsing Fever.
	2. J-1623	1-Encephalitis. 2-Poliomyelitis.	1-Encephalitis. 2-Poliomyelitis.	1-Hemorrhagic Diathesis with Pontine and Subdural Hemorrhage.	1-Relapsing Fever.
	3. J-1642	1-F.U.O.	1-Relapsing Fever.	1-Relapsing Fever.	1-Relapsing Fever.
	4. J-1659	1-F.U.O.	1-Possible Typhoid with Acute Hepatic Necrosis.	1-Fulminant Infectious Hepatitis.	1-Relapsing Fever.
	5. J-1992	1-Infectious Hepatitis	1-Relapsing Fever.	1-Relapsing Fever.	1-Relapsing Fever.
II	6. J-1627	1-Dysentery.	1-Bacillary Dysentery	1-Bacillary Dysentery. 2-Splenomegaly, Etiology Undetermined.	1-Bacillary Dysentery. 2-Relapsing Fever.
	7. J-1689	1-Pneumonia.	1. Jaundice, Etiology Undetermined. 2-CNS Hemorrhage and Bleeding Tendency from Impaired Liver Function.	1-Salmonella enteritidis Endocarditis with Septicemia. 2-Cerebral and Splenic Embolism. 3-Hepatitis with Jaundice.	1-Salmonella Endocarditis with Septicemia. 2-Relapsing Fever.
	8. J-1719	1-F.U.O.	1-Typhoid Fever. 2-Empyema.	1-Salmonella Septicemia.	1-Salmonella Septicemia. 2-Relapsing Fever.
	9. J-2284	1-F.U.O.	1-Salmonella Septicemia.	1-Salmonella Septicemia.	1-Salmonella Septicemia. 2-Relapsing Fever.
III	10. J-1966	1-F.U.O.	1-Relapsing Fever.	1-Salmonella Septicemia.	1-Salmonella Septicemia.
	11. J-1985	1-F.U.O.	1-Relapsing Fever. 2-Acute Yellow Atrophy. (Herxheimer ?)	1-Salmonella Endocarditis.	1-Salmonella Endocarditis.

F.U.O. = fever of unknown origin.

are indicated in Table IV. Cultures were not performed in all cases, but pathologic findings, including bacterial stains, ruled out coexisting bacterial infection in cases of group I.

TABLE
Clinical Data in 11 Cases

	CASE	AUTOPSY NUMBER	NATIONALITY	AGE	ADMISSION SYMPTOMS	DURATION PRIOR TO ADMISSION (DAYS)	HOSPITAL COURSE (DAYS)	JAUNDICE	CNS AND MENINGEAL SIGNS	RASH, HEMORRHAGES
I	1.	J- 563	Amer.	23	Malaise, Chills, Fever, Anorexia Headache.	3	1	None	None	None
	2.	J- 1623	Korean	30	Chills, Headache, Generalized Aching, Fever.	7	3	None	Coma, Impaired Hearing.	None
	3.	J- 1642	Korean	47	Moribund	No History	1	None	Stupor, Spastic Meningismus.	Ecchymoses Site of Injection.
	4.	J- 1659	Korean	21	Chills, Fever, Anorexia, Headache.	10	2	Marked	Coma	None
	5.	J- 1992	Amer.	20	Fever, Anorexia, Abdominal Pain, Headache.	14	1	Mild	Meningismus, Disorientation.	Red Cells in Spinal Fluid.
II	6.	J- 1627	Korean	Unk.	Headache, Chills, Diarrhea, Anorexia, Abdominal Cramps.	14	17	None	None	None
	7.	J- 1689	Korean	21	Anorexia, Cough, Generalized Aching, Difficult Hearing.	3	8	Moderate	Stiff Neck, Coma.	Red Cells in Spinal Fluid, Hemorrhages in Oral Cavity.
	8.	J- 1719	Korean	21	Malaise, Anorexia, Evening Fever, Cough, Chest Pain.	15	12	Mild	None	None
	9.	J- 2284	Korean	19	Diarrhea, Anorexia, Backache, Chills, Fever.	8	1	Marked	Clouded Sensorium, Meningismus, Impaired Hearing	Macular Skin Rash.
III	10.	J- 1966	Chinese	21	Bloody Diarrhea, Chills, Headache, Arthralgia, Fever.	8	3	Marked	None	Petechiae and Ecchymoses, Skin.
	11.	J- 1985	Korean	22	Diarrhea, Headache, Nosebleed, Fever, Aching.	10	20	Marked (Late)	Delirium (Late)	Hemorrhagic Skin Rash.

III
of Relapsing Fever

HEPATO-MEGALY	SPLENO-MEGALY	CLINICAL DIAGNOSIS	BORRELIA SMEARS	WBC	URINE	SPINAL FLUID	SPECIFIC TREATMENT	MISCELLANEOUS
No	Yes	1-Ruptured Spleen. 2-Hemoperitoneum.	None	None	2+ to 4+ Alb. OCC RBC.	None	None	
Yes	No	1-Encephalitis. 2-Poliomyelitis.	None	None	None	8-13 Lymphs, 0→1+ Pandy, Xanthochromia.	None	
Yes	No	1-Relapsing Fever with CNS Involvement.	Positive	25,000 (N-76, L-26)	2+ ALB, Many RBC, Bile Neg., Urobil.+1/20	None	1-Penicillin, 2,100,000 u. 2-Chloramphenicol 5 gm.	Bleeding Time - 2 min. Clotting Time - 4 min. Clot Retracted Thymol Turbidity-15 Cardiolipin - 2+
Yes	Yes	1-Hepatic Necrosis. 2-? Typhoid Fever.	None	None	None	None	None	Stool Culture: Negative
Yes	No	1 Relapsing Fever with Meningismus.	Positive	58,000 Metamyelos-17 Myelos-12	Bile Present.	500 RBC, 7 WBC, Borrelia Present.	Penicillin, 900,000 u. in oil.	
No	Yes	1-Bacillary Dysentery. 2-Toxic Granulocytopenia.	None	11,500 (N-76, L-22 M2) 5,650 (N-4, L-90 M6) Late	Tr. ALB.	None	1-Sulfadiazine -18 gm. 2-Chloramphenicol-12 gm.	Widal Negative Stool Culture: Sh. flexneri IV
No	Yes	1-Jaundice, Etiology Undetermined. 2-CNS Hemorrhage. 3-Pneumonia.	None	None	None	Bloody	None	Widal "H" 1:640 "O" 1:320 Bleeding Time 5 min. Clotting Time 19 min. Rumple Lead Negative Autopsy Culture: S. enteritidis, Blood, Spleen, Stool, Mesenteric Lymphnodes, Bile, Urine, Meninges.
Yes	Yes	1-Typhoid Fever. 2-Empyema.	None	None	None	None	Penicillin	Widal "H" 1:1,280 Autopsy Culture: S. cholera-suis, Stool, Lung, Empyema, Spleen, Bile, Lymphnodes.
Yes	Yes	1-S. enteritidis Septicemia.	Negative	16,600	2+ ALB., Few RBC, Bile Pos. Urobil.+1/16	None	Chloramphenicol, 3 gm.	Blood, Urine, Stool, Cultures: S. enteritidis. Autopsy Cultures: S. enteritidis, Blood, Bile, Spleen.
Yes	No	1-Overwhelming Relapsing Fever.	Positive	5,200 to 9,200	None	None	Mapharsen, 0.060 gm.	Autopsy Cultures: S. enteritidis, Blood, Bile.
No	Yes	1-Relapsing Fever. 2-Acute Yellow Atrophy (Herxheimer ?)	Positive	7,000	3+ ALB., Many WBC, Bile Pos.	None	1-Mapharsen 0.060 gm. 2-Penicillin 2,400,000 u.	Widal Negative Icteric Index 51 and 94, Vandenbergh. Direct +, Indirect 6.0 mgm.% and 8.1 mgm.% Cephalin Floc. 4+ Autopsy Cultures: S. enteritidis, Blood, Spleen.

Clinical Data

The pertinent clinical data are summarized in Table III. None of the patients in this series presented a classical history of recurrent febrile episodes. It is understandable, therefore, that relapsing fever was unsuspected in most instances. Sudden onset of chills, fever,

Table IV

Method of Diagnosis of Relapsing Fever

			Smears			Warthin-Starry stains, tissues													
			Blood (clinical)	Spinal fluid (clinical)	Blood (necropsy)	Heart	Lung	Spleen	Liver	Brain	Kidney	Pancreas	Adrenal gland	Thyroid gland	Lymph node	Bone marrow	Testis	Prostate	Intestine
I	1	J-563	O	O	+	−	−	+	+	O	+	−	−	−	−	−	−	−	O
	2	J-1623	O	O	O	+	+	+	+	+	+	+	+	O	+	+	+	+	+
	3	J-1642	+	O	O	+	+	+	+	+	O	+	O	+	O	O	O	O	+
	4	J-1659	O	O	O	+	+	+	+	+	+	+	+	O	+	+	+	+	+
	5	J-1992	+	+	+	+	+	+	+	+	−	+	+	O	O	O	O	O	+
II	6	J-1627	O	O	O	O	−	+	−	−	−	O	−	O	−	O	O	O	−
	7	J-1689	O	O	O	O	O	+	−	O	O	O	O	O	O	O	O	O	O
	8	J-1719	O	O	O	O	−	+	−	−	−	O	O	O	O	O	O	O	−
	9	J-2284	−	O	O	−	−	+	−	−	−	−	−	−	−	−	−	−	−
III	10	J-1966	+	O	−	−	−	−	−	−	−	O	−	−	−	O	−	−	−
	11	J-1985	+	O	−	−	−	−	−	−	−	O	O	O	−	O	−	−	−

+ = Spirochetes present.
− = Spirochetes absent.
O = Not examined.

anorexia, and headache was the history usually obtained. In patients who had a complicating Salmonella or Shigella infection (groups II and III), diarrhea also was frequent, whereas only one of the 5 in group I complained of "loose stools." Exanthems and jaundice also were observed more frequently when an enteric infection complicated relapsing fever.

Pathologic Findings

General

The observations made on external examination corroborated the physical findings, e.g., icterus of mild to intense degree in 7 patients, hepatosplenomegaly in 7, and macular rash or petechiae in 3 with Salmonella septicemia. Patients with uncomplicated relapsing fever presented no gross evidence of serosal inflammation, whereas serositis was a feature in 4 of the 6 cases with complicating Salmonella septicemia or shigellosis. This was manifested by empyema and fibrinous

pericarditis in cases 8 and 11, respectively, and perisplenitis in cases 6, 7, and 11. In the patient with splenic rupture (case 1) approximately 5 l. of clotted blood were in the left upper quadrant of the abdomen, surrounding the site of the rupture.

Visceral changes were predominantly in the heart, spleen, liver, kidneys, and brain. Although some pathologic changes were common to all groups, other changes were peculiar to a single group. Typical of group I were more severe degrees of nonsuppurative myocarditis, softening in splenic follicles, diffuse hepatitis, and hemorrhages of the central nervous system. In group II with Salmonella septicemia, the changes were related to the septicemia and included inflammation of serosal membranes, endocarditis, septic infarction of the spleen, and abscess formation in kidney and myocardium. Enteritis was a constant finding. In several cases, softening in the splenic follicles, characteristic of relapsing fever, was noted in addition to the septic splenic infarcts. In neither of the cases in group III, in which therapy apparently controlled the relapsing fever, were similar lesions observed. Diffuse myocarditis, common in cases of group I, was observed in one case of group II but in neither of the cases of group III.

Heart

No unusual gross pathologic changes were noted in the hearts in group I. Weights of the organ varied from 300 to 325 gm. Histologically, however, interstitial myocarditis was present in 4 of the 5 cases (Fig. 1). Interstitial mononuclear cell infiltrates, consisting of lymphocytes, infrequent plasma cells, and occasional polymorphonuclear leukocytes, were characteristically diffuse, but more prominent about vessels and within the epicardium. In 2 cases, spirochetes were demonstrated within vessels, myocardial fibers, and interstitial tissues. In some vessels the spirochetes existed as clumps of tangled organisms (Figs. 2 and 3). Myocarditis was more intense in those hearts in which spirochetes were demonstrated. Similar myocardial changes were observed in only one case in group II (case 9) and in none of group III.

In the presence of Salmonella septicemia, endocarditis and suppurative myocarditis were observed in 2 cases (cases 7 and 11). Vegetations along the free edges of the commissures and intercommissural fissures of the mitral valves were granular and friable, and measured 0.2 to 0.3 cm. in diameter. Histologically, they were composed of fibrin with enmeshed polymorphonuclear leukocytes and bacteria; early organization was evident at the base of these lesions. Old scar-

ring of the valve was not observed. Gram-negative bacilli were demonstrated in the vegetations with the Goodpasture stain, and Salmonella organisms were cultured from the heart's blood in both cases.

Lungs

The lungs presented varying degrees of congestion, edema, and bronchopneumonia in 10 of the 11 cases. No observable differences between the groups were noted. In case 8, however, the *Salmonella choleraesuis* infection had caused lobar pneumonia and empyema, organisms being cultured from both lung and pleural fluid.

Spleen

Splenomegaly was present in all cases. The weight of the spleens in group I varied from 300 to 500 gm. There were no capsular alterations except in the case with splenic rupture. On cut section, however, yellowish foci, varying from 0.1 to 0.3 cm. in diameter, were observed in 3 of 5 spleens. Generally, these miliary foci were more prominent in the peripheral areas of the organ, being somewhat indefinite or obliterated in the mushy central portions. Grossly, they resembled minute areas of suppuration, but actual liquefactive necrosis was not present. Microscopically, these foci proved to be "miliary abscesses" involving follicles and perifollicular splenic tissue (Figs. 4 and 5). Within these splenic lesions were polymorphonuclear leukocytes, fragmented nuclear débris, and macrophages containing nuclear fragments and polymorphonuclear leukocytes. Increased numbers of polymorphonuclear leukocytes and plasma cells were present in the intervening pulp.

With silver impregnation large numbers of spirochetes were demonstrated in the peripheral portions of the "miliary abscesses" and adjacent splenic pulp (Fig. 5). They were seen as tangled, intertwining masses forming a prominent lacy network about the periphery of the lesion. Numerous single and fragmented forms were interspersed throughout this network of spirochetes and frequently they were seen end to end. Usually they were 10 to 12 μ in length, with five to seven loose undulations and tapering ends. Giemsa stains failed to demonstrate spirochetes, but Warthin-Starry preparations counterstained with eosin revealed intracytoplasmic as well as extracellular spirochetes (Fig. 6).

The weights of the spleens in groups II and III varied from 275 to 1,400 gm., averaging 712 gm. Well demarcated, irregular, wedge-shaped areas of softening, usually subcapsular, were present in 5 of

the 6 cases. Hereafter these will be called infarcts, although arterial occlusion was not demonstrable. In cases 6 and 8, miliary abscesses were interspersed throughout the remainder of the spleen. Follicular softening was absent in both cases of group III. Gross perisplenitis, as indicated by fibrinopurulent exudate, was observed in 2 cases in group II and one of group III.

Microscopically, the infarcts consisted of a peripheral zone of congested splenic pulp circumscribing an exudative zone, which contained numerous polymorphonuclear leukocytes, cellular débris, spirochetes, and bacteria. Central to this zone, the parenchyma was completely necrotic (Fig. 7). In 2 cases follicular abscesses were observed in the splenic pulp adjacent to the areas of infarction (Fig. 9). With Warthin-Starry stains, spirochetes were demonstrated most commonly in the periphery of the infarcts in combination with plump, rod-shaped bacteria (Fig. 8). In the central portions of the smaller infarcts spirochetes were seen only occasionally while plump, rod-shaped bacilli usually occurred in large numbers (Fig. 10). These bacilli were gram-negative with Goodpasture's stain. Vascular occlusion was not demonstrated microscopically in any of these spleens with infarcts, but endophlebitis of trabecular vessels was common.

Liver

Mild to moderate hepatitis was present in 4 of the 5 patients of group I. In the fifth case (case 1) only mild fatty metamorphosis was observed. Grossly, these livers were slightly enlarged; on section, lobular markings and sinusoidal patterns were obscured. Microscopically, foci of necrosis of liver cells were predominantly midzonal or in the central portions of the lobule (Fig. 11). In these necrotic foci the remaining liver cells were interspersed with cellular infiltrates composed of lymphocytes, occasional polymorphonuclear leukocytes, and eosinophils. In other parts of the lobule, Kupffer cells were prominent within widened and usually bloodless sinusoids. Portal areas were unaltered except for minimal cellular infiltrates composed of lymphocytes and infrequent eosinophils. Evidence of extrahepatic bile duct obstruction was not noted. With Warthin-Starry stains, many spirochetes were observed within sinusoids, parasinusoidal spaces, and liver cells in cases 2 and 4 (Fig. 12). Hepatitis was most severe in cases with marked jaundice. In cases of groups II and III, foci of liver cell necrosis were much less prominent or absent, but in several cases the sinusoids were filled with large numbers of polymorphonuclear leukocytes and lymphocytes. Portal areas were essentially unaltered except

for dilatation of bile duct radicals and periductal fibrosis in the patient with *Clonorchis sinensis* infection (case 8). Spirochetes were not demonstrated in the liver in any of the cases in groups II and III.

Kidneys

The average combined weight of the kidneys in group I was 340 gm.; in group II, 340 gm.; and in group III, 450 gm. Grossly, the kidneys in group I were pale in 3 instances and bile-stained in 2 (cases 3 and 5). Histologically, glomerular changes could not be detected, but in the 3 kidneys described as pale, there were granular, reddish brown casts in distal convoluted and collecting tubules, with minimal associated epithelial necrosis characterized by eosinophilia of the cytoplasm, pyknosis of nuclei, and disruption of epithelial continuity. Tubular necrosis was more prominent in 2 cases of group II (cases 7 and 9). In 3 additional cases of groups II and III (cases 8, 9, and 11) focal cortical abscesses were present.

Brain

Hemorrhages of the central nervous system were observed frequently in cases of group I. Massive subdural and less extensive pontine, medullary, and intracerebral hemorrhages were present in 2, and foci of hemorrhage, measuring 0.2 to 0.4 cm., in the floor of the fourth ventricle and cerebral cortex in 2 others. There was diffuse hyperemia of the leptomeningeal vessels. Histologically, no cellular reaction or neuronal change of the brain tissue was observed adjacent to areas of hemorrhage. Leptomeningeal vessels contained settled aggregates of leukocytes, but endothelial and mural changes were absent. Warthin-Starry stains demonstrated spirochetes within cerebral vessels, in Virchow-Robin spaces, and in areas of hemorrhage in 3 cases (Fig. 13). They appeared as tangled masses in the brain tissue immediately surrounding the hemorrhages, and as single organisms or in clumps within the hemorrhages. In the fourth case (case 4) spirochetes were demonstrated in an area of focal arachnoiditis (Fig. 14). In one of the 2 cases with massive cerebral hemorrhage (case 3), spirochetes were present only in cerebral tissues. Silver preparations were negative for spirochetes in all cases of groups II and III, although subcortical petechiae were seen in temporal and parietal lobes in 2 of these cases.

The pancreas, adrenal glands, bladder, prostate, testes, and thyroid and pituitary glands revealed no significant gross or microscopic changes. Petechiae and subacute inflammatory changes of the intesti-

nal mucosa and submucosa with associated lymphadenitis of mesenteric lymph nodes were seen in patients with enterocolitis in groups II and III. Enterocolitis was not observed in cases of group I. Moderate myelocytic hyperplasia of bone marrow was present in 2 cases (cases 2 and 4).

Cause of Death

In the 5 cases of group I, death was attributable solely to relapsing fever: as the result of splenic rupture (case 1), intracranial hemorrhages (cases 2 and 3), leptomeningitis (case 5), and hepatitis (case 4). In the remaining 6 cases there was a complicating bacillary infection. In 5 of the latter, death was considered a direct result of Salmonella infection. Acute Salmonella endocarditis with septic embolization was present in cases 7 and 11, Salmonella septicemia with hepatitis in cases 9 and 10, and Salmonella pneumonia with empyema in case 8. The only patient with bacillary dysentery (case 6) apparently died of toxemia due primarily to relapsing fever, for at the time of his death the dysentery had been brought under control.

Discussion

Relapsing fever in Korea is a remarkably different disease from that encountered in certain parts of the United States. In this country it is a mild tick-borne disease, which rarely, if ever, kills the patient. Korean relapsing fever is a louse-borne disease with a high incidence of serious complications and a significant mortality rate.

Because American physicians had no experience with the disease and because it so frequently was associated with other infections, notably Salmonella septicemia, clinical and pathologic diagnosis was often difficult. In the 5 cases in which death was due to relapsing fever not complicated by Salmonella or Shigella infection (group I), a clinical diagnosis was established in only 2 instances. In none of the 4 cases of group II was relapsing fever suspected by the clinician or prosector at the time of necropsy. In the 2 cases of group III, on the contrary, relapsing fever was diagnosed and treated, but Salmonella septicemia was unsuspected until established by post-mortem cultures.

The association of relapsing fever and typhoid-paratyphoid fever or bacillary dysentery was a frequent observation in epidemics of relapsing fever during the late 19th century and early 20th century. The concept of bilious typhoid proposed by Griesinger,[11] in 1864, was subsequently expanded to include relapsing fever. As early as 1884, Lübimoff[1] observed micrococci in abscesses and infarcts of the spleen and other viscera in fatal relapsing fever, and concluded that pyemia

was a frequent complication of the epidemic disease in Russia and Europe.

During and after World War I, mixed infections with relapsing fever were observed commonly in Russia and Europe. In a study of 190 Serbian soldiers with relapsing fever during World War I, Hunter[2] observed coexistent cholera in 25, typhoid fever in 14, and dysentery in 3. In 1923, Kulescha and Titowa[3] described a combined infection of relapsing fever and paratyphoid fever during an epidemic in Leningrad, Russia. Although the bacteria isolated from their cases were of the typhoid-paratyphoid group, they were unable to classify them definitely. These organisms differed in their biologic and serologic characteristics from the types of paratyphoid bacilli known at that time. Because the organisms produced suppurative foci in numerous viscera, including heart, spleen, and kidney, the authors suggested that louse-borne relapsing fever in earlier European and Russian epidemics may have been complicated by pyemia of similar nature. Iwaschenzoff[12] also noted that many cases of relapsing fever in the Leningrad epidemic of 1922 differed from typical cases. They were characterized by the shorter interval between relapses, progressive enlargement of the spleen and liver, diarrhea, intense jaundice, and higher mortality rate. Bacteria, resembling *Bacillus paratyphosus B* and the bacillus of Danysz, were isolated from urine, blood, bile, feces, and from suppurative foci in various organs. The suppuration, involving multiple viscera, was like that described by Kulescha and Titowa. Many years later Hicks[13] reported that the organisms recovered in these epidemics were *Salmonella hirschfeldii* and *Salmonella moscow*.

Similar observations have been made in China[4,14] and Egypt.[15] Of 337 Chinese patients with relapsing fever studied by Chung and Chang,[4] 15 had associated Salmonella septicemia and 16 had coexisting bacillary dysentery. El-Ramly[15] reported that typhoid fever and dysentery were frequent in patients with relapsing fever during the Egyptian epidemic of 1944 to 1946.

With few exceptions, the incidence of Salmonella septicemia in most reported epidemics of relapsing fever is not known, either because bacteriologic studies were not carried out or because the actual number of cases in which cultures were made is not recorded. The data available indicate that the incidence varies with the epidemic and, as a rule, less than 10 per cent of the patients have coexisting Salmonella septicemia or bacillary dysentery. Since the number of patients studied bacteriologically among the 175 with relapsing fever in Korea during

1950 and 1951 is unknown, the incidence of Salmonella septicemia and dysentery in this epidemic cannot be estimated. Similarly, accurate figures on relative mortality rates in relapsing fever and relapsing fever complicated by Salmonella septicemia or bacillary dysentery are not generally available or accurate because of the unknown incidence of salmonellosis and shigellosis in most epidemics. However, in a few epidemics, cases with coexisting Salmonella septicemia were reported to carry a higher mortality. In Chung and Chang's[4] series of 337 cases of relapsing fever, a mortality rate of 67 per cent was observed in 15 cases of relapsing fever complicated by Salmonella septicemia, in contrast to a mortality of 3.4 per cent in the remaining 322 patients. Iwaschenzoff[12] reported a mortality rate of 63 per cent in another group with Salmonella infection, compared with an over-all mortality of 17 per cent in the entire series.

This dual infection has been ascribed to the transmission of Salmonella organisms and Borrelia by infected lice. Chung and associates[16,17] have shown that body lice obtained from their patients harbored *Salmonella enteritidis* as well as Borrelia. They suggested that the practice of cracking lice between the teeth or crushing them between fingernails, a habit of many who are lousy, may be responsible for transmission of Salmonella organisms in cases of louse-borne relapsing fever. There was no direct evidence that this was the mode of transmission in the Korean cases. At the time these cases of relapsing fever occurred, a much larger number of Salmonella and dysentery infections were encountered also in prisoners of war.[10,18-20] It seems more plausible that Salmonella organisms were transmitted in the usual manner.

The prominent clinical and pathologic features of those cases of relapsing fever with salmonellosis were intense jaundice, diarrhea, rash, septic splenic infarction, and suppuration in multiple viscera. Distinctive findings in relapsing fever uncomplicated by Salmonella septicemia or shigellosis included jaundice, leukemoid reactions, interstitial myocarditis, follicular changes in the spleen, central nervous system hemorrhages, and spontaneous rupture of the spleen.

Jaundice, interstitial myocarditis, central nervous system hemorrhages, and follicular changes in spleen were found in both groups of patients. However, with the exception of jaundice, these features were more characteristic of cases of relapsing fever uncomplicated by salmonellosis. By contrast, significant diarrhea, septic splenic infarction, and suppuration in multiple viscera were confined to those cases of relapsing fever with salmonellosis.

Although jaundice was generally more severe in those cases with Salmonella septicemia, it was observed in both groups of cases and did not have specific differential value. In one case of each group, jaundice was of such severity that fulminating hepatitis was suspected clinically (Table III). Jaundice has been described as a common finding in relapsing fever. Ludlow[6] reported that it occurred in one half of 300 cases in Korea, and Chung and Chang[4] observed it in 84, or 24.9 per cent, of 337 cases in China. In patients with severe jaundice, *S. enteritidis* infections commonly were present. Ludlow, and Chung and Chang considered jaundice a poor prognostic sign. Robertson[21] stated that jaundice was an inconstant sign in epidemics in China and that it varied both in incidence and severity. He concluded that jaundice was of no prognostic import. Wolff[7] described jaundice in 15 of 134 cases of relapsing fever in Chinese soldiers in Burma during World War II, and El-Ramly[15] observed jaundice in 43 of 139 fatal cases of relapsing fever in Egypt during 1945.

The occurrence of jaundice in relapsing fever probably depends on a number of factors, among which are the severity of relapsing fever, the time at which specific therapy is initiated, the presence of Salmonella septicemia, and the age of the patient. Intensely jaundiced patients in this series either had severe relapsing fever without benefit of early therapy or a complicating Salmonella septicemia.

Diarrhea and skin rash associated with intense jaundice in patients with relapsing fever are sufficiently distinctive features to warrant investigation of coexistent enteric disease. Macular rash was observed in 3 patients with Salmonella septicemia. Chung and Chang[4] described rash in 117 of 337 cases of relapsing fever, but observed that it was more prominent and frequently purpuric in patients with *S. enteritidis* infections. Robertson,[21] on the other hand, found rash infrequent in relapsing fever.

The predominantly purpuric nature of the rash of relapsing fever has been noted by several authors. Babes[22] estimated that it occurred in that form in 50 per cent of patients in the Rumanian epidemic of 1915. El-Ramly[5] described a purpuric rash in 42 of 139 fatal cases of relapsing fever in the Egyptian epidemic. Although Chu, Deitrick, and Chung[23] observed rash in Chinese children with relapsing fever, they ascribed it to the bites of lice. These observations suggest that the rash in relapsing fever is characteristically purpuric. Erythematous, macular, desquamative rash is characteristic of salmonellosis.

Septic infarctions of the spleen were observed in 6 patients in this series, 5 of whom had coexistent Salmonella septicemia and one, bacil-

lary dysentery. None of the patients with uncomplicated relapsing fever had splenic infarcts. Splenic infarction has been described frequently in fatal cases of relapsing fever in other epidemics. Kulescha and Titowa[3] reported septic splenic infarcts in 128 of 268 cases. Splenic abscesses had developed in 37 patients and 27 of them had ruptured to produce peritonitis. Material from 19 of these spleens was cultured, and n-paratyphoid bacilli were recovered in 10. El-Ramly[15] observed splenic infarction in 75 of 3,000 consecutive cases; 52 were classified as simple and 23 as septic. Cultures were not reported. In the same epidemic, however, Nasr[24] reported isolation of *Staphylococcus aureus* in only one of seven splenic cultures. Our data indicate that positive cultures of spleen may be obtained frequently despite antibiotic therapy.

In the cases of group II, spirochetes were observed only within areas of splenic infarction or in follicular abscesses of the spleen. None were found in other tissues. Occasional spirochetes and larger numbers of bacilli were present in the central acellular and necrotic portions of the infarct. Here they were generally present as single extracellular organisms; rarely as tangled masses of spirochetes seen in the follicular lesions adjacent to the areas of infarction. Since mercurial or antibiotic therapy of various types was employed in all of these cases, the absence of spirochetes in tissue other than spleen and their presence in ischemic tissues of the spleen suggest that the antibiotic agent failed to reach the spirochetes in these ischemic areas in sufficient concentration to accomplish their destruction.

Another pathologic feature confined to those patients with coexistent Salmonella septicemia was suppuration in multiple viscera. Pyemia in patients with relapsing fever and coexistent Salmonella septicemia has been a common observation in other epidemics.

The most characteristic pathologic change of relapsing fever is the follicular "abscess" observed in the spleen. The distinctive nature of this lesion is illustrated by Cleland's[25] report of a fatal case in Australia. Although he observed follicular changes in the spleen at necropsy, their etiology remained obscure until he read Russell's[26] description of follicular softening in spleens of relapsing fever. Sections of the spleen, taken from the gross museum specimen and prepared by the silver method 11½ years later, were positive for spirochetes.

The follicular "abscesses" were found in 6 of the 11 cases of the present series; 4 of 5 with uncomplicated relapsing fever and 2 of 6 with associated Salmonella septicemia. The association of the follicular

changes with septic infarctions of the spleen in 2 patients of the latter group is indicative of the double infections which occurred among these patients. Follicular lesions of the spleen were first described in detail by Nikiforoff,[27] who observed, in experimental infections in monkeys, that spirochetes were concentrated within splenic follicles while they were disappearing from the blood just before the crisis. After the crisis, spirochetes disappeared even from the splenic lesions. He regarded the focal splenic lesions as a manifestation of relapsing fever and distinct from suppuration. Russell[26] also described the splenic lesions in some detail and commented on the peculiar selective polymorphonuclear leukocytic response to the spirochetes of relapsing fever. Nasu[28] observed crescentic areas of follicular softening which he interpreted as early lesions. Similar crescentic foci were observed in only one case in this series (case 1). The course of this patient's illness was brief, for he died of exsanguination from a ruptured spleen. The evidence suggests that the duration of his illness was insufficient for complete development of the "follicular abscess" which had reached only the "half moon" stage described by Nasu.

In only one patient of group I was there no evidence of follicular softening in the spleen. In this patient therapy was administered early, and death resulted from extensive cerebral hemorrhage. All tissues except the brain were negative for spirochetes. Presumably spirochetes were destroyed in the spleen early in the course of the disease and formation of the splenic lesion was aborted. The absence of spirochetes in tissues of the 2 patients treated with mapharsen, who subsequently died of salmonellosis, also suggests that early therapy may prevent formation of the splenic lesion. It seems unlikely that infarction or superimposed septicemia would obliterate the follicular lesion or destroy the spirochetes, since typical areas of follicular softening were observed in 2 cases with infarction of the spleen, and spirochetes and bacteria were demonstrated together in both cases.

Although jaundice was more frequent and more intense in relapsing fever complicated by Salmonella septicemia, it was observed also in uncomplicated relapsing fever. In fact, a clinical diagnosis of acute hepatic necrosis was made in case 4 and the initial necropsy diagnosis was fulminant hepatitis. Although hepatitis has not often been reported, jaundice is fairly frequent in fatal cases of relapsing fever. Nasu[28] observed hepatitis in 9 of 18 Japanese with relapsing fever, and El-Ramly[15] described hepatitis in Egyptian relapsing fever.

Interstitial myocarditis, observed in 4 of 11 cases of this series, was more pronounced in the 2 cases in which spirochetes were demonstrated

in the myocardium. Myocarditis seldom has been observed, but Schuster[29] found interstitial round cell infiltrates in half of the hearts of 28 European children dying of relapsing fever.

Hemorrhages of the central nervous system frequently interfered with clinical and pathologic interpretation in the cases of this series (Table II). Brain involvement in humans has been described in both louse-borne and tick-borne relapsing fever by Babes,[22] Jahnel and Lucksch,[30] Hunter,[2] Belezky and Umanskaja,[31] and Dewar and Walmsley.[32] In one of Babes' fatal cases, the meninges were thickened and hemorrhagic; spirochetes were demonstrated in meningeal vessels. Jahnel and Lucksch described spirochetes within vessels in the meninges, cerebral cortex, and subcortical white matter of the cerebellum in 2 fatal cases. Belezky and Umanskaja observed spirochetes in the gray matter of the cerebrum and medulla, especially in the pia-arachnoid, in 5 cases of fatal relapsing fever. Phagocytosis of spirochetes by glial elements had occurred, but parenchymal cells had undergone no significant alterations. Mononuclear cell infiltrates, chiefly lymphocytes and plasma cells, were present only in the pia-arachnoid.

The pathologic changes of the brain in the 2 patients with massive cerebral hemorrhage in this series were similar to those described by Belezky and Umanskaja.[31] Spirochetes were seen in the pia-arachnoid and in the parenchyma adjacent to hemorrhages. In neither location, however, was phagocytosis of spirochetes observed. Of considerable interest was case 3 in which spirochetes were seen only in brain tissue (Table IV). Belezky and Umanskaja also recorded cases in which spirochetes were demonstrable only in brain tissue. A similar localization in the brain is observed occasionally in experimental animals infected with Borrelia and treated with penicillin.[33] The cerebral changes described by Martinez-Baez and Villasana[34] in experimental infections correspond to the focal changes observed in human cases without massive hemorrhages. Animals sacrificed during febrile attacks showed focal perivascular hemorrhages, measuring 0.2 to 0.3 cm. in diameter, in the cerebral cortex without neuronal changes; animals sacrificed at intervals after the initial attacks showed essentially no cerebral alteration except for discrete lymphocytic infiltrates of the pia-arachnoid.

The demonstration of spirochetes in spinal fluid sediment by dark-field microscopy, as in case 3, is seldom achieved, although the infectivity of spinal fluid has been established by inoculation into splenectomized squirrels.[35]

Spontaneous splenic rupture, which occurred in one of the 11 cases,

is unusual in relapsing fever. El-Ramly[15] reported 4 cases of splenic rupture among 139 patients who died of relapsing fever during the Egyptian epidemic. In each of his cases, a capsular tear was observed at the upper pole of the spleen. In only one of these spleens was infarction described, and this was distinct and apart from the site of the tear.

Summary

The clinical and pathologic observations made in a series of 11 necropsied cases of Korean louse-borne relapsing fever are presented with special attention to the association of this spirochetal disease with Salmonella and Shigella infections. Two cases, both unassociated with salmonellosis or dysentery, occurred in American soldiers. All others involved Korean or Chinese prisoners of war. In 5 cases death was attributable solely to relapsing fever, as the result of intracranial hemorrhages in 2 patients and of meningitis, hepatitis, and splenic rupture in the remaining 3. Of 6 patients with a complicating bacillary infection, 5 were considered to have died as a result of Salmonella infection. One patient with shigellosis died of toxemia due to relapsing fever after his dysentery had been controlled.

Follicular splenic abscesses, interstitial myocarditis, intracranial hemorrhages, and hepatitis with focal necrosis were the most distinctive features of Borrelia infection. Enteritis, splenic infarcts, and evidence of suppuration were pathologic expressions of complicating bacillary infection.

The association of salmonellosis and dysentery with louse-borne relapsing fever has been reported in many epidemics widely separated in time and space. It has not been reported in the mild sporadic cases of tick-borne relapsing fever observed in the United States. American physicians called to duty in parts of the world where louse-borne relapsing fever is encountered should be alert to the possibilities of these dual infections. Recognition of such combinations of infectious diseases is of therapeutic and prognostic importance as well as of academic interest.

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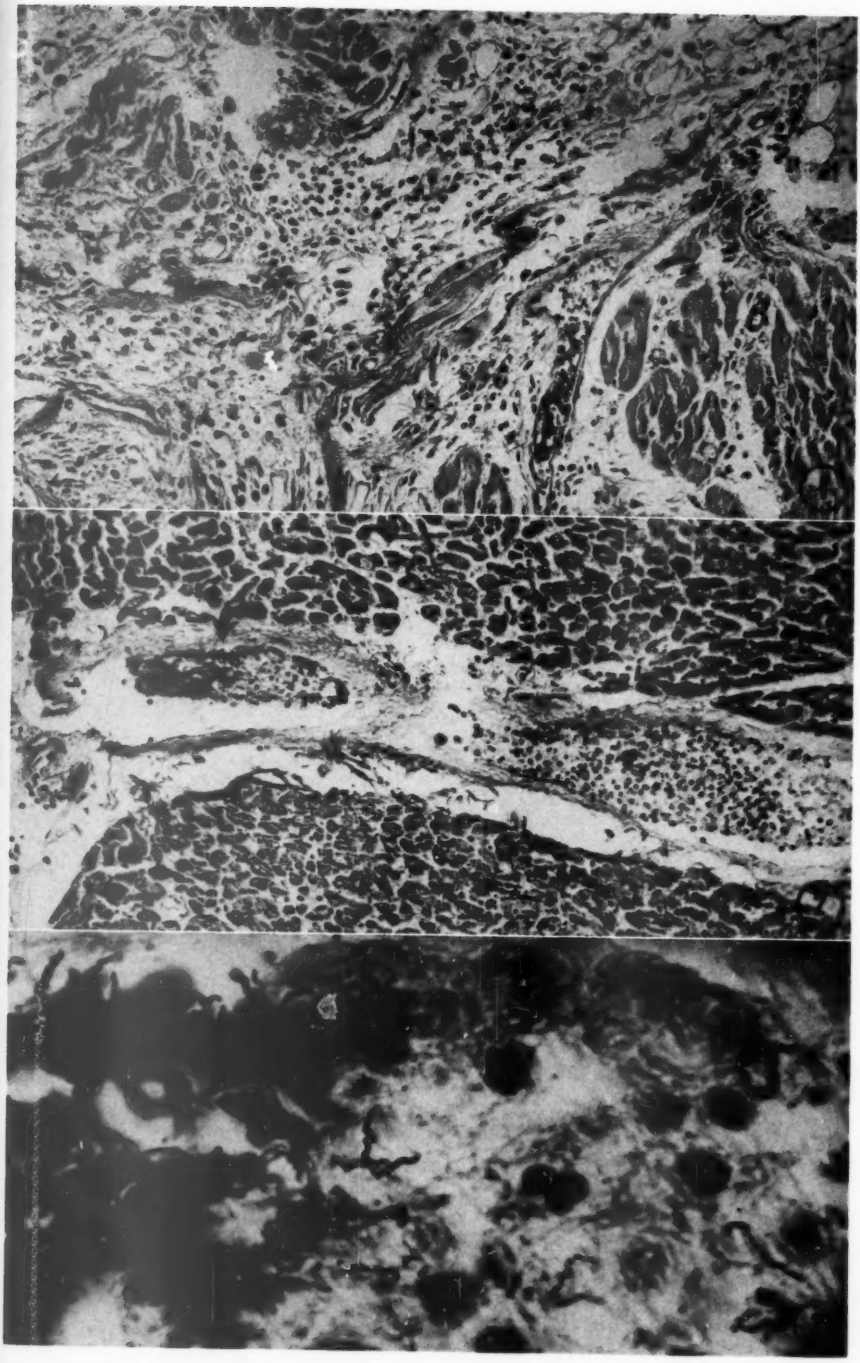
LEGENDS FOR FIGURES

FIG. 1. Case 2. Interstitial mononuclear cell infiltrate of myocardium and epicardium. Hematoxylin and eosin. × 184.

FIG. 2. Case 2. Tangled mass of spirochetes in branch of coronary vessel, indicated by arrow. Warthin-Starry method. × 165.

FIG. 3. Case 2. Higher magnification of the area indicated in Figure 2, illustrating the intertwining mass of spirochetes within coronary vessel. Warthin-Starry method. × 1,780.



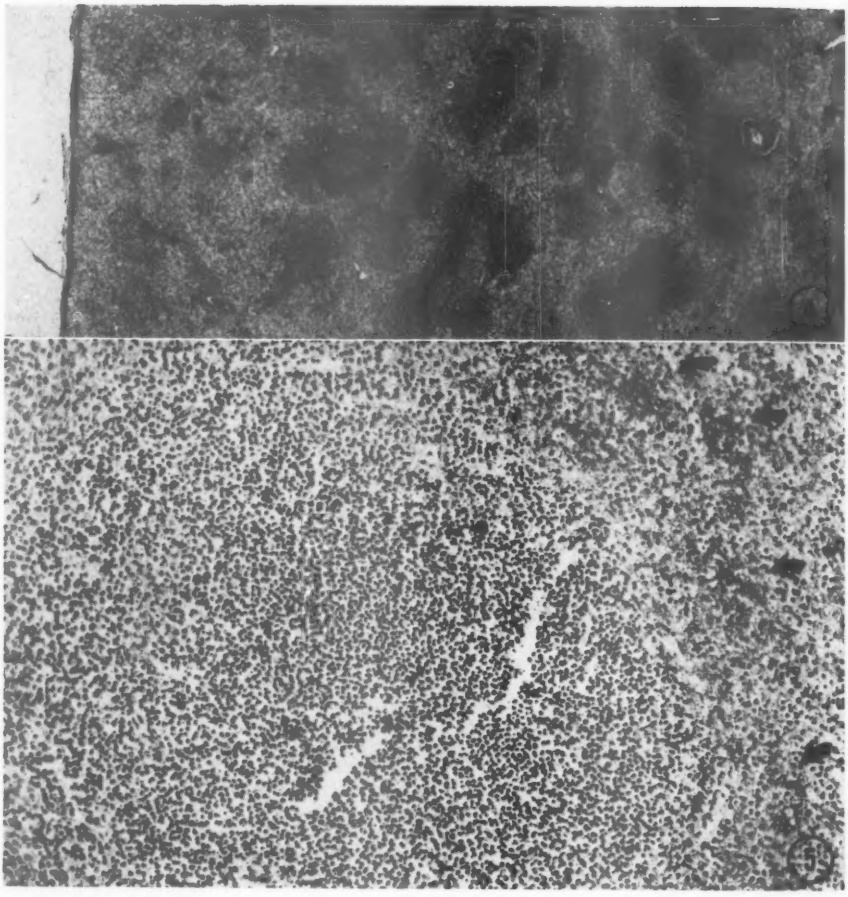


FIG. 4. Case 2. Miliary foci of softening in spleen. Hematoxylin and eosin method. × 19.

FIG. 5. Case 2. Miliary "abscess" of spleen. The lacy, black pattern to the right of the abscess represents tangled masses of spirochetes. Warthin-Starry method. × 142.

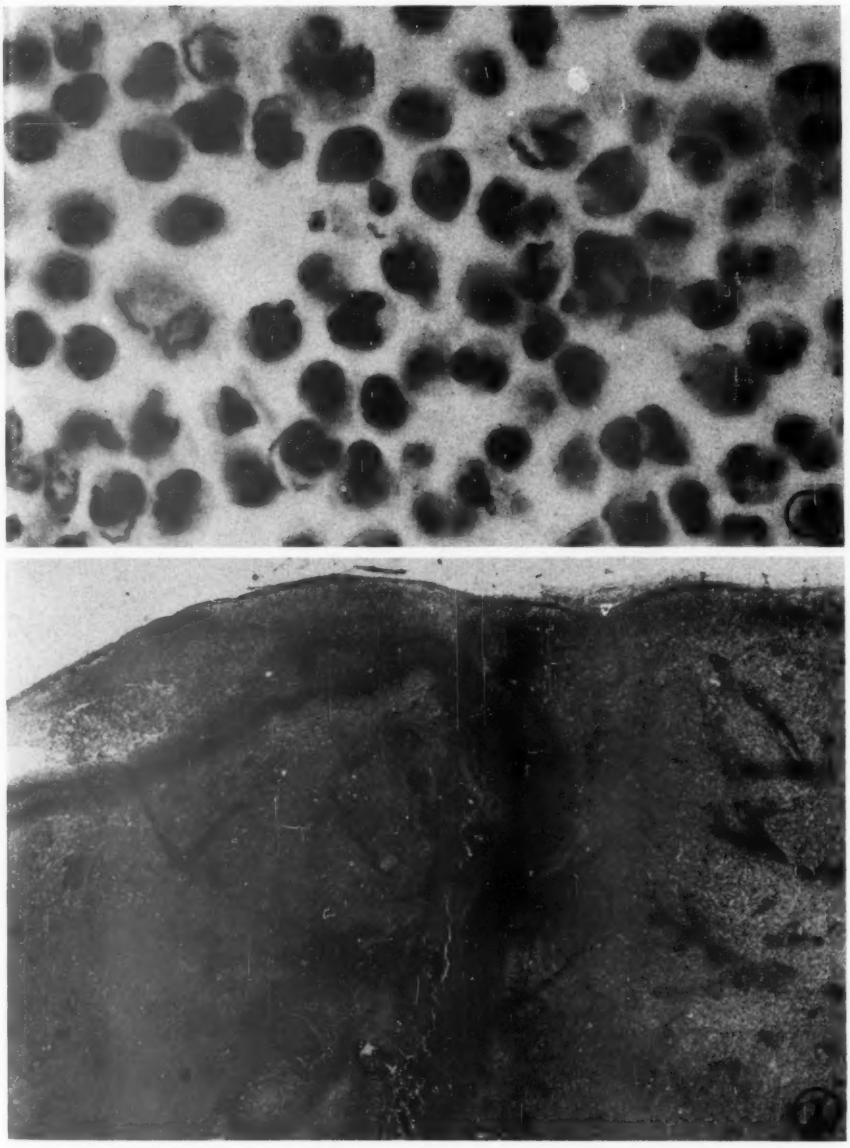


FIG. 6. Case 2. Center of follicular abscess of spleen, illustrating intracytoplasmic spirochetes in polymorphonuclear leukocytes and macrophages. Warthin-Starry method, counterstained with eosin. × 1,600.

FIG. 7. Case 6. Infarct of spleen with central acellular zone of ischemic necrosis. The area indicated in the center of the field is shown at higher magnification in Figure 8. Warthin-Starry method. × 19.

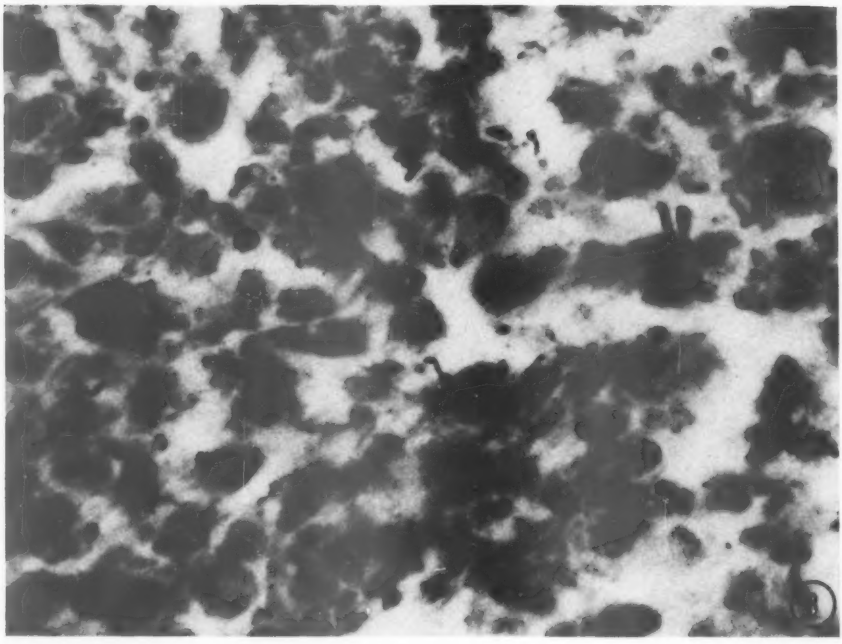


FIG. 8. Case 6. Periphery of infarct shown in Figure 7 (arrows) showing admixture of bacilli and spirochetes. Some spirochetes are within macrophages and many are disintegrating. Warthin-Starry method. × 1,746.

FIG. 9. Case 9. Infarct of spleen (A) with adjoining foci of suppuration represented by lighter areas (B). The area in center of infarct (arrows) is shown in greater magnification in Figure 10. Warthin-Starry method. × 19.

FIG. 10. Case 9. Central portion of infarct in Figure 9, indicated by arrows, showing plump, rod-shaped bacilli. Spirochetes are not present. Warthin-Starry method. × 1,780.

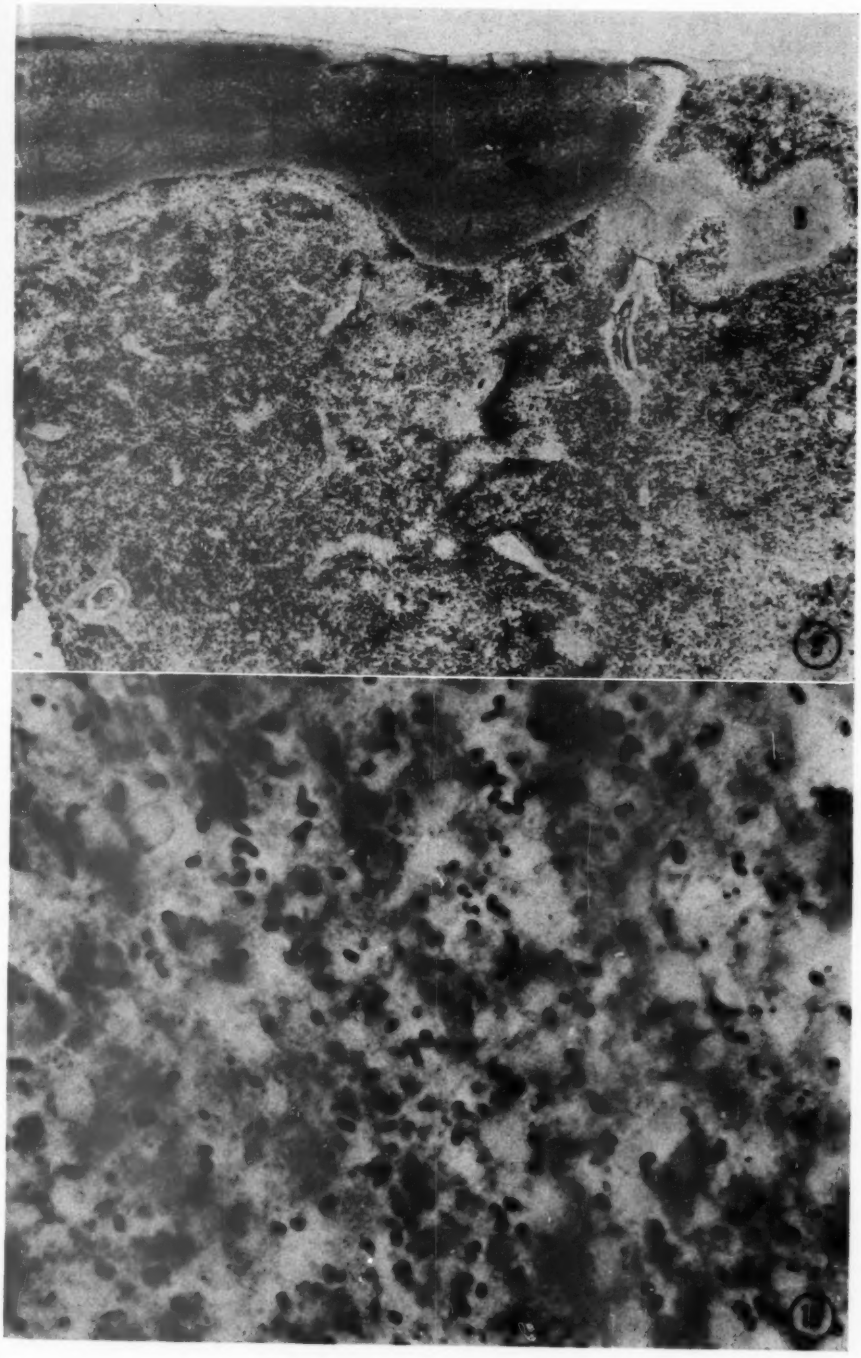
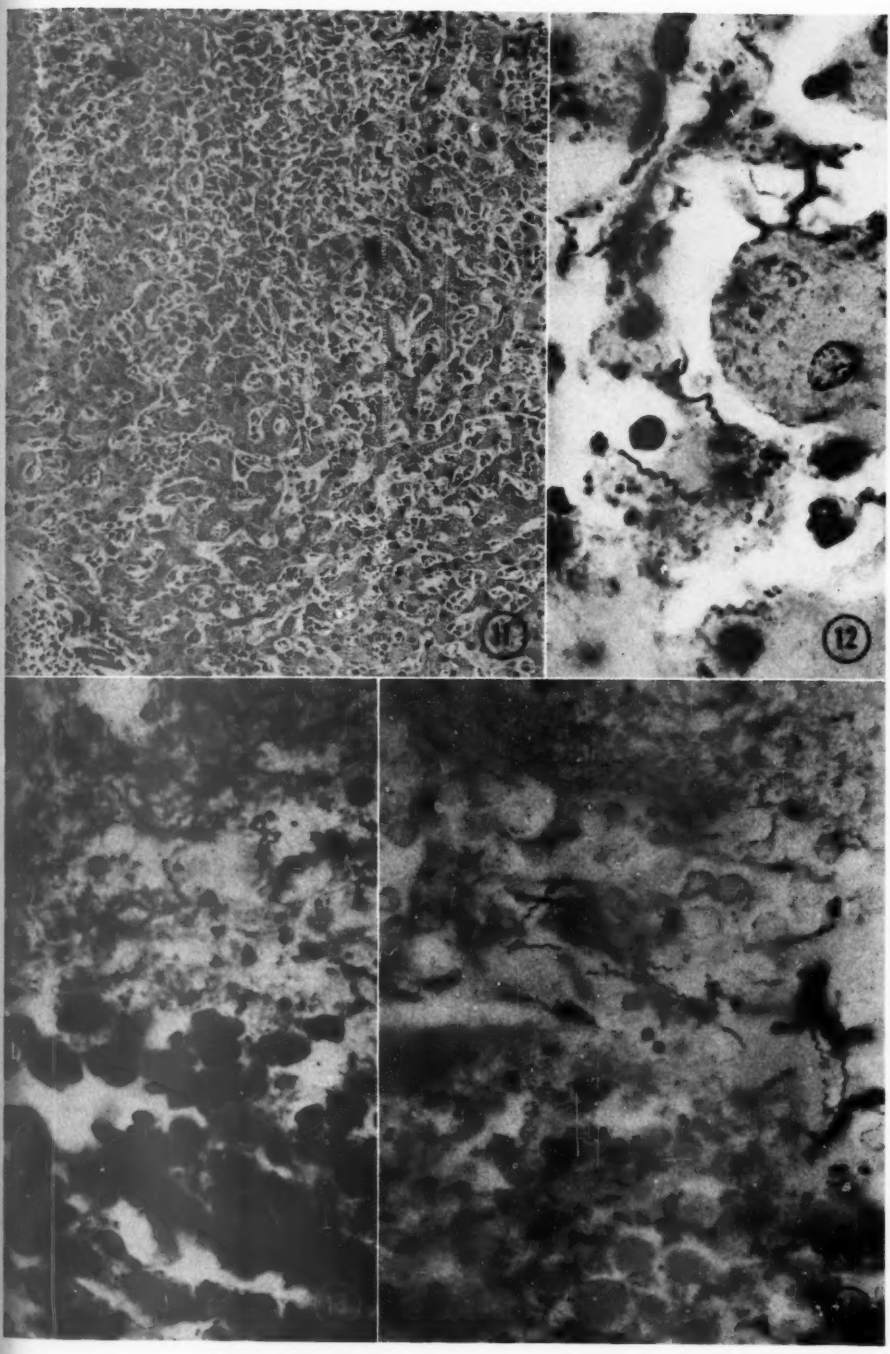


FIG. 11. Case 4. Centrilobular necrosis of liver, with mononuclear cell infiltrate. The sinusoidal architecture is disorganized and hepatic cells are necrotic in an area (arrows) adjacent to the central vein (CV). Mononuclear cells are present throughout the sinusoids and in the portal areas (PA). Hematoxylin and eosin stain. × 165.

FIG. 12. Case 2. Spirochetes in sinusoids of liver. Warthin-Starry method. × 1,780.

FIG. 13. Case 4. Spirochetes in perivascular spaces of leptomeninges. Warthin-Starry method. × 1,600.

FIG. 14. Case 5. Tangled masses of spirochetes in cerebral tissue surrounding area of hemorrhage. Warthin-Starry method. × 1,780.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HISTOPATHOLOGY OF AMINO ACID DEFICIENCIES

IV. TRYPTOPHAN *

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The morphologic effects of protein deficiency and specific tissue reactions to individual amino acid deficiencies have interested investigators for some time. The present report is the fourth in a series dealing with the response of tissues to such deficiencies. Studies of the effects of tryptophan deficiency have shown that the lack of this essential amino acid leads to alopecia, cataract formation, corneal vascularization, defective dentition, fatty liver, inhibition of bone growth, alteration of both cardiac and visceral muscle, and gonadal atrophy or reproductive failure.[1-9] Since the techniques employed in the present study differ from those reported previously by others and since additional effects were observed, the results are presented.

MATERIAL AND METHOD

Young male Sprague-Dawley rats of a similar age and weight were divided at random into three groups. One group of 4 rats (normal control) was fed a purified diet consisting of nineteen crystalline amino acids, vitamins, sucrose, cottonseed oil, and the necessary minerals and salts as described by Rose, Oesterling, and Womack.[10] A second group of 10 rats (deficient) received the same diet, complete in every respect, except for the total omission of tryptophan. The caloric value of the missing tryptophan was supplied by additional sucrose. The third group (semistarved control) was pair-fed the complete diet so that their consumption of food was no greater than the amount consumed by each of the deficient animals. All rats were kept in individual cages and, with the exception of the pair-fed group, were fed ad libitum. At the termination of the experimental period of 30 days, all rats were sacrificed by exsanguination under ether anesthesia. At necropsy, the following tissues were removed: liver, spleen, pancreas, intestine, stomach, kidney, adrenal gland, abdominal aorta, ureter, testes, ventral prostate, seminal vesicles, thyroid gland, esophagus, lymph node, thymus, heart, lung, skeletal muscle, urinary bladder, brain, pituitary

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body, and tibia. Only the testes and adrenal and pituitary glands were weighed at necropsy.

All tissues, except the pituitary glands, were fixed in Bouin's solution and stained with hematoxylin and eosin. In addition, blocks of liver and one adrenal gland were fixed in 10 per cent neutral formalin, embedded in gelatin, sectioned on the freezing microtome, and stained with either osmic acid or Sudan black B. The pituitary glands were fixed in Zenker-formol solution and stained by the aldehyde-fuchsin (AF) and periodic acid-Schiff (PAS) methods. These methods were developed by Halmi[11] and Purves and Griesbach[12,13] to demonstrate and differentiate the thyrotrophic and gonadotrophic basophils of the anterior pituitary body. The employment of these methods in amino acid deficiency studies was described in our earlier reports.[14-16] Other pituitary sections were stained with acid fuchsin for study of the acidophils.

Observations

Weight loss was a consistent feature of the tryptophan-deficient rats, although the range of loss was wide. In the semistarved control rats, weight gain or loss, as the case might be, was minimal. All organs in the deficient animals were smaller grossly than in the pair-fed controls. Table I lists the initial and final body weights and weights of the pituitary and adrenal glands and of the testes of each rat.

Pituitary Body. Cellular alteration of the pituitary glands of deficient rats was confined to the anterior lobe. The nuclear size and cytoplasmic content of the acidophils were greatly reduced (Fig. 3), but the general distribution of these cells was normal. By application of the AF and PAS methods, it was seen that the thyrotrophic-hormone-producing basophils were intact, while the presence of gonadotrophic-hormone-producing basophils could not be demonstrated (Figs. 8 and 9). In the semistarved control rats, the acidophils were smaller than normal but the reduction in size was not as severe as in the deficient rats (Fig. 2). Partial inanition had no effect upon the AF-positive thyrotrophic basophils (Fig. 6). However, there was some reduction in the staining capacity of the gonadotrophic cells, but these cells were easily recognized (Fig. 7).

Testes and Accessory Structures. Microscopically, the seminiferous tubules were greatly reduced in diameter and neither spermatids nor spermatozoa were present (Fig. 11). Numerous degenerating cells were free in the tubular lumina. Spermatogonia and some early spermatocytes were observed, but development did not continue. The testicular interstitial cells were flat. The prostatic acini were extremely

small and in both prostates and seminal vesicles the epithelium was atrophic and non-secretory and the interstitial connective tissue much increased (Fig. 13). The accessory sex glands had every appearance of suffering from the lack of Leydig cell hormonal support.

The seminiferous tubules of the semistarved controls were smaller than normal but spermatogenesis was active, although spermiogenesis lagged (Fig. 10). The prostatic acini were reduced in size from

TABLE I

Comparison of Body Weights and Organ Weights of Tryptophan-Deficient, Pair-Fed Semistarved Control and Normal Control Rats

	Rat no.	Body weight: Start	Final	Change	Organ weight: Adrenal glands (2)	Testes (2)	Pituitary body
		gm.	gm.	gm.	mg.	mg.	mg.
Tryptophan deficient	65-1	52	37	−15	20.6	179.8	3.4
	65-2	48	31	−17	18.2	123.8	1.8
	65-3	35	35	0	17.0	235.4	2.4
	65-4	48	35	−13	15.6	127.0	1.6
	65-5	42	35	− 7	21.0	129.8	1.8
	65-6	41	36	− 5	20.0	246.6	2.8
	65-7	48	32	−16	18.4	124.8	3.0
	65-8	52	35	−17	18.6	119.2	2.6
	65-9	53	40	−13	19.6	287.4	2.8
	65-10	50	33	−17	19.2	135.8	2.8
Semistarved control	65SC-1	51	50	− 1	23.4	192.0	2.8
	65SC-2	48	53	+ 5	22.8	237.6	3.4
	65SC-3	55	58	+ 3	25.2	408.2	3.0
	65SC-4	48	53	+ 5	20.6	380.6	3.2
	65SC-5	48	58	+10	21.8	514.2	2.6
	65SC-6	52	58	+ 6	21.2	270.8	3.2
	65SC-7	55	61	+ 6	29.4	590.6	2.8
	65SC-8	57	55	− 2	24.2	308.8	3.6
	65SC-9	44	53	+ 9	22.4	293.2	3.2
	65SC-10	55	59	+ 4	25.2	641.4	3.4
Normal control	60-65-1	51	186	+135	38.0	2437.0	7.8
	60-65-2	53	189	+136	33.6	2389.8	7.4
	60-65-3	57	191	+134	47.2	2841.8	8.6
	60-65-4	40	176	+136	37.4	2455.8	8.4

the normal but not as much as in the deficient rats (Fig. 12). Both the prostate and seminal vesicles were reduced in secretory activity. The epithelial cells appeared to be lacking full hormonal support from the testicular interstitial cells.

Liver. Examination of paraffin and frozen sections of the livers of the deficient rats revealed lipidosis of the hepatic cells (Fig. 15). In general, the degree was moderate, but in one rat it was heavy (Fig. 14). There appeared to be no relationship between the distribution of the lipidosis and either the vascular elements of the liver or any specific portion of the hepatic lobule.

Thymus. In addition to being grossly involuted, the thymus appeared to have undergone a degenerative process resembling lipoidal degeneration. Differentiation of the cortical and medullary areas was lacking. Necrotic areas were scattered throughout the remains of the organ, and spindle-shaped clefts, which have been described as being characteristic of cholesterol deposits, were very conspicuous (Fig. 21). The true nature of the contents of these clefts could not be determined, since all thymic tissue had been prepared for paraffin sectioning. Giant cells were associated with the necrotic areas and the needle-like clefts.

By contrast, the thymuses of the semistarved control rats were smaller than normal but the cortical and medullary zones were intact and no necrotic areas, crystalline deposits, or giant cells were observed (Fig. 20).

Heart. In all tryptophan-deficient rats some form of myocardial damage was observed. Not all of the injuries were identical but they appeared to represent a number of stages in a process of degeneration and repair. Lesions of one type, which was considered to be an early stage, exhibited karyolysis and cytoplasmic vacuolation (Fig. 24). These areas usually were small. Another lesion, which may have been a later stage in the process, showed hyalinization and cloudy swelling of the injured muscle (Fig. 25). Two other lesions, which were examples of early healing, showed replacement of muscle fibers by loose areolar stroma (Fig. 26) and invasion of the necrotic area by young fibroblasts (Fig. 27).

Adrenal Glands. The fascicular zone of the adrenal cortex was narrowed considerably in the deficient rats. The relative widths of the glomerulosal and reticular areas were within the normal range. Examination of frozen sections stained with Sudan black B showed that the lipid content of the cortex either was limited principally to the zona fasciculata (Fig. 18) or was depleted almost completely from the entire cortex (Fig. 19). The medulla of the deficient rats was unaffected.

In the semistarved control animals the adrenal cortex was narrowed slightly and contained large amounts of lipid in all zones (Fig. 17). No appreciable alteration in the proportional widths of the cortical zones was observed. The medullary area was unaltered.

Bone. Microscopic examination of decalcified sections of the tibia showed that bone growth was retarded far more in the deficient rats (Fig. 23) than in their pair-fed controls (Fig. 22). In the deficient rats, not only were the epiphyseal cartilage plates narrower, but no newly formed bony trabeculae were present, as they were in the semi-

starved controls. In the latter, the epiphyseal cells were arranged in columns characteristic of growing bone.

Pancreas. The pancreatic acinar cells of the deficient rats were decreased in size and the acini were small. These cells were deeply basophilic and zymogen granules were scanty. Islet tissue was unaltered and well distributed.

Other Organs and Tissues. Examination of other organs revealed only a gross reduction with corresponding reduction in the size of their components.

Comment

In studies of this type one must consider the rôle played by partial inanition as well as the part played by the deficiency, since anorexia is common in all experiments dealing with amino acid deficiencies. It is not unreasonable, therefore, to assume that the effects of tryptophan deficiency were superimposed upon the effects of partial inanition. However, there were some histologic changes in the tryptophan-deficient rats which did not occur in the pair-fed controls. These certainly were due to the lack of dietary tryptophan.

Reduction in the size of the pituitary acidophils, complete absence of demonstrable gonadotrophic basophils, atrophy of the testes and accessory structures, thymic involution, and interruption of bone growth have all been described in our studies of phenylalanine, threonine, and histidine deficiencies.[14-16] That the lack of these individual amino acids had no demonstrable effect upon the pituitary thyrotrophic basophils was observed also in the earlier and present studies. On the other hand, lipidosis of the hepatic cells, myocardial degeneration, and the presence of crystalline deposits with giant cell invasion of the thymus were not observed previously in the experiments on deficiency of single amino acids, and were present following the lack of tryptophan.

Gonadal atrophy or reproductive failure following tryptophan deficiency has been reported by Adamstone and Spector;[1] Albanese and Buschke;[4] Brown, Wilkening, and Schweigert;[5] Keller;[6] and Albanese, Randall, and Holt.[9] The pituitary bodies were not examined in these experiments. From my observations, it seems probable that gonadal atrophy resulted directly from the lack of gonadotrophic hormonal support, which was a result of the depletion of the gonadotrophic basophils brought about by the lack of tryptophan. The loss of this hormonal support was responsible also for Leydig cell deterioration; and, in turn, the prostate and seminal vesicles were affected.

The severe loss of cytoplasm and the reduction in the size of the

pituitary acidophils of the tryptophan-deficient animals were similar to the effects of phenylalanine, threonine, or histidine deficiencies. The lack of these amino acids resulted in failure of somatotrophic hormone supply. Reduction in the size of these cells in the semistarved control rats was not as great and, in these rats, the acidophils were able to maintain some hormonal support. Comparison of the epiphyseal cartilages and of the body and organ weights of the two groups supports this view.

Cole and Scott[7] referred to thyroid changes in their study of tryptophan-deficient rats. In the experimental work which I am reporting the histologic appearance of the thyroid glands of the deficient animals was well within the normal range. The absence of dietary tryptophan had no effect upon the presence or function of the thyrotrophic basophils. The normal appearance of the thyroid glands supports the view that they were receiving sufficient hormonal support from the pituitary body.

The relationship between the adrenal cortex and the site of ACTH formation is not clear. Cushing,[17] in his observations of pituitary basophilism, suggested that the pituitary basophilic cells were the most likely site of ACTH formation. In the guinea-pig, D'Angelo, Gordon, and Charipper[18] observed that starvation gave rise to adrenal hyperplasia which they believed was due to an increase in adrenotrophin secretion. This was accomplished by an increase in basophils and chromophobes and a fall in acidophils. The site of the source of adrenotrophin has been placed at the pituitary acidophil by Heinbecker and Rolf.[19] These workers reported that the adrenal cortex remained normal after sectioning of the infundibular stalk and fibers from the paraventricular nuclei, while the number of basophils decreased and the number of acidophils remained normal. Finerty and Brinseno-Castrejon,[20] after performing unilateral adrenalectomy in rats, observed compensatory hypertrophy of the remaining adrenal gland and a marked increase in the percentage of acidophils. These investigators also concluded that the acidophils were the source of ACTH. Martin[21] and Reese, Koneff, and Akimoto[22] are in agreement that the acidophilic cells of the pituitary body regress in size and staining capacity after total adrenalectomy, but they are not in agreement with respect to the basophils. From the present experiment it is difficult to place the source of adrenotrophin, since depletion of one type of basophil and regression in size of the acidophils occurred. The loss in cortical lipid content and reduction in the fasciculata may have

been due to the lack of adrenotrophic stimulation by the pituitary body or to direct action of the deficiency upon the cortex.

Adamstone and Spector[1] observed cardiac muscular changes in tryptophan-deficient rats which they described as "shredding." Myocardial lesions appear to be related specifically to the lack of tryptophan, since no effects of this nature were observed by us in the pair-fed controls or in those rats which had previously been fed diets deficient in phenylalanine, threonine, or histidine. It is not regarded as probable that infection was responsible for the cardiac lesions. There is the possibility, however, that the deficient rats may have been more susceptible to an infectious agent than were the semistarved controls.

Adamstone and Spector[1] described degeneration of visceral muscle fibers. We observed no such alteration of smooth muscle. Cole and Scott,[7] and Brown, Wilkening, and Schweigert[6] reported reduction in the diameter of skeletal muscle fibers. Such alteration in the size of skeletal muscle fibers was observed in the present study, but no particular significance was attached to this. Since the entire muscular architecture was diminished, one could expect the component fibers to be reduced in size.

My observations with regard to lipidosis of the liver as a result of tryptophan deficiency are in accord with those of other investigators.[1,7] Apparently this accumulation of fat was brought about by some interference with the normal metabolism of the liver. It is of interest to note that lipidosis of the hepatic cells was not observed by me in rats placed on other single amino acid deficiencies so far studied.

Thymic atrophy has been a common finding in our studies on amino acid deficiency. However, crystalline deposition, presumably of cholesterol, with the accompanying foreign body reaction (macrophage invasion and giant cell formation) had not been observed in our earlier studies of amino acid deficiencies nor reported by others interested in tryptophan deficiency. Examination of lymph nodes and spleen showed no such effects, and why the involuted thymus should be selected as the site for such changes cannot be answered at this time.

Summary

The total lack of dietary tryptophan resulted in some tissue changes which were similar to the histologic effects of phenylalanine, threonine, or histidine deficiencies. These were regression in the size of the anterior pituitary acidophils, complete depletion in the anterior pituitary gonadotrophic basophils, atrophy of the testes and accessory

structures, and thymic involution and interference with somatic growth. There were some histopathologic effects, however, which were not observed previously and which appear to have resulted directly from the total lack of tryptophan. These include lipidosis of hepatic cells, myocardial lesions, and crystalline deposition in an already involuted thymus.

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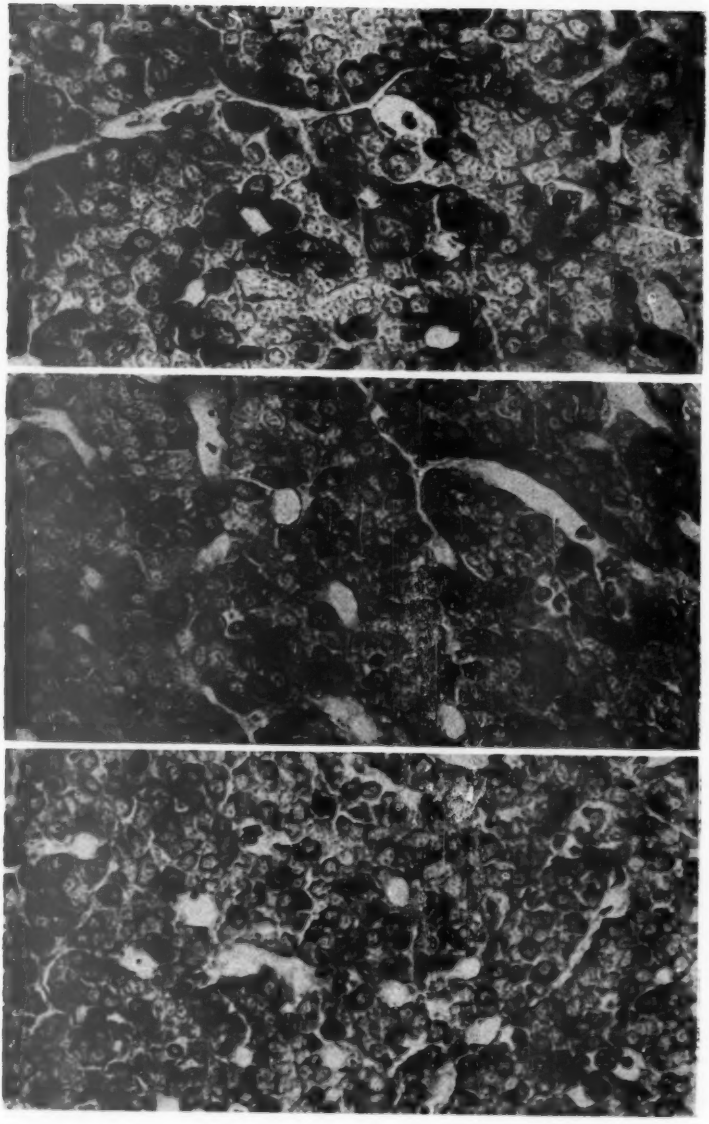
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Anterior pituitary body of a normal control rat, stained with acid fuchsin for identification of the acidophils. × 430.

FIG. 2. Anterior pituitary body of a semistarved control rat, stained with acid fuchsin. The acidophils are smaller than normal. × 430.

FIG. 3. Anterior pituitary gland of a tryptophan-deficient rat, stained with acid fuchsin. The acidophils are very small, with reduction in nuclear size and cytoplasmic content. × 430.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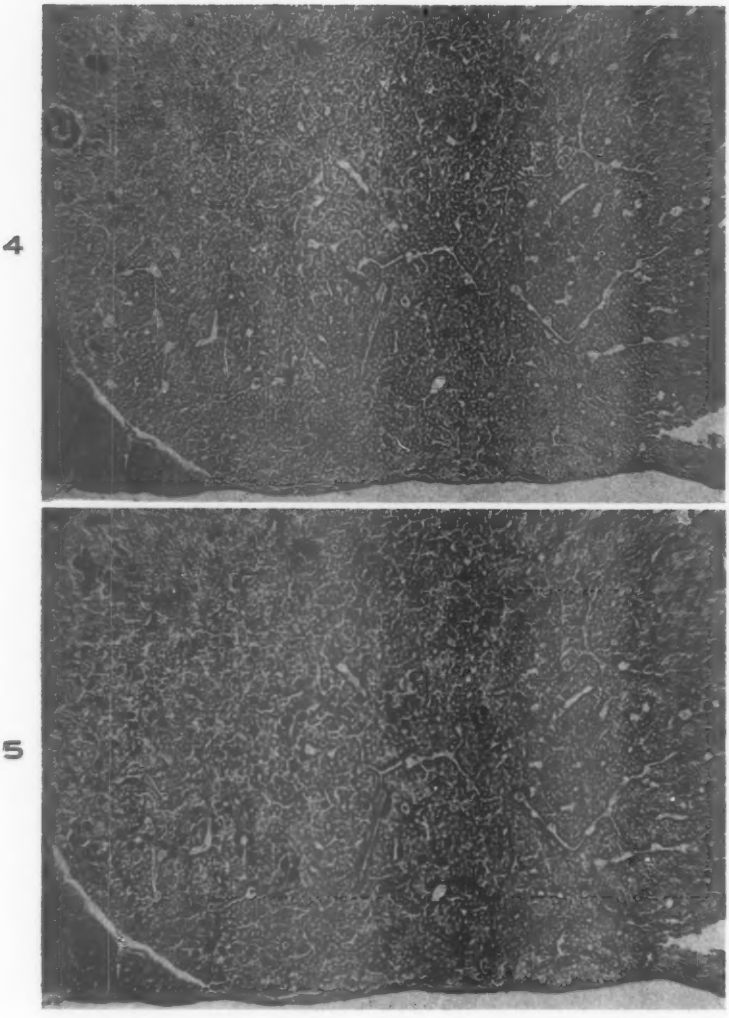
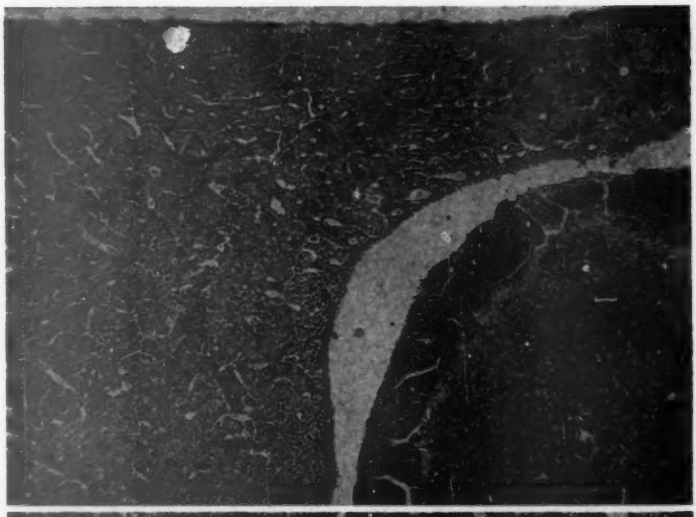


FIG. 4. Anterior pituitary body of a normal control rat, stained by the aldehyde-fuchsin (AF) method for demonstration and identification of the thyrotrophic basophils. The AF-positive dark cells are the thyrotrophic cells. × 100.

FIG. 5. The same section and field shown in Figure 4, restained by the periodic acid-Schiff (PAS) method. All basophils are PAS-positive. The additional cells, stained in this section but which are not apparent in Figure 4, are the gonadotrophic basophils. × 100.



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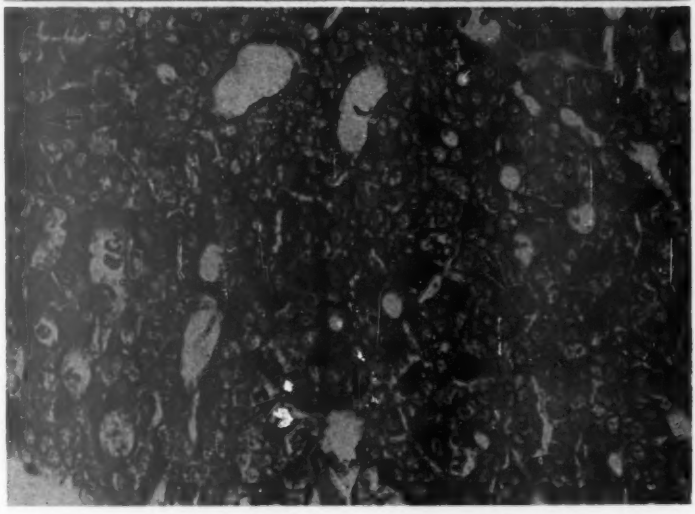

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FIG. 6. Anterior pituitary body of a semistarved control rat, stained by the AF method to show the thyrotrophic cells. × 100.

FIG. 7. The same section shown in Figure 6 after restaining by the PAS method. Because the gonadotrophic basophils are fainter than normal and difficult to illustrate photographically under lower magnification, they are shown here at a higher magnification; some are indicated by arrows. The very dark cells are thyrotrophic basophils. × 430.

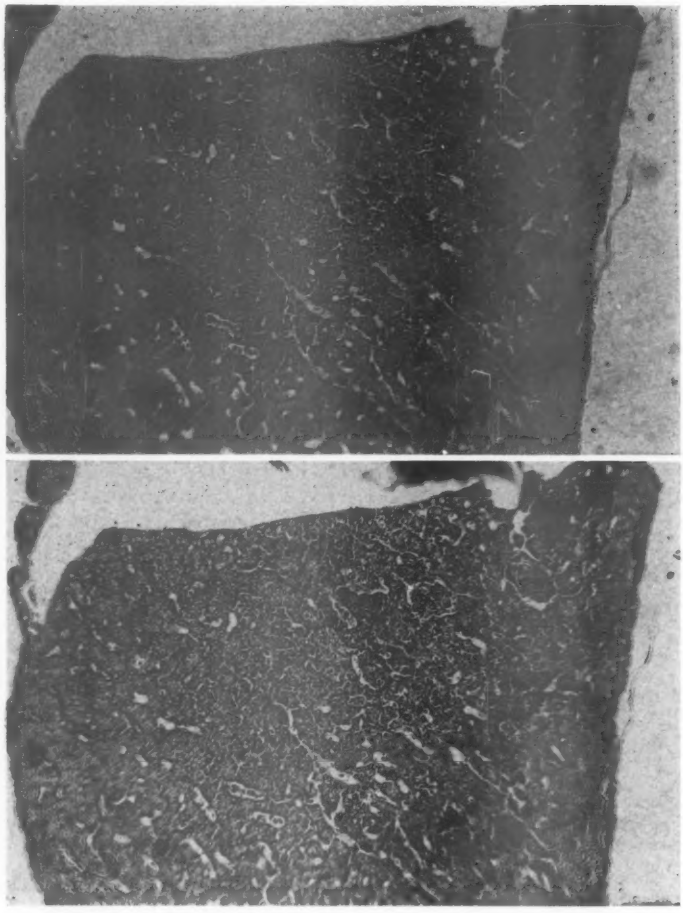


FIG. 8. Anterior pituitary gland of a tryptophan-deficient rat, stained by the AF method. The dark thyrotrophic basophils are apparent. × 100.

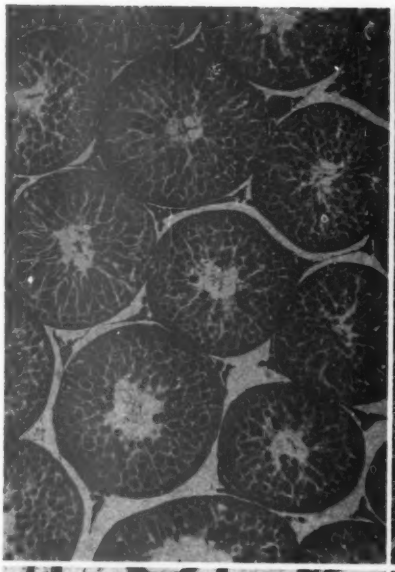
FIG. 9. The same section and field shown in Figure 8, restained by the PAS method. Only those cells which were AF-positive in Figure 8 are PAS-positive, demonstrating the complete absence of gonadotrophic basophils. × 100.

FIG. 10. Testis of a semistarved control rat. × 100.

FIG. 11. Testis of a tryptophan-deficient rat. × 100.

FIG. 12. Ventral prostate of a semistarved control rat. × 100.

FIG. 13. Ventral prostate of a tryptophan-deficient rat. × 100.



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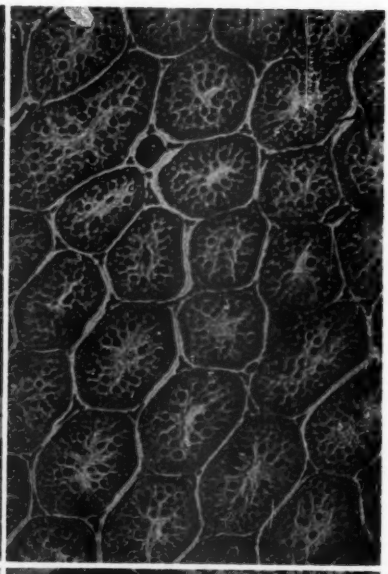

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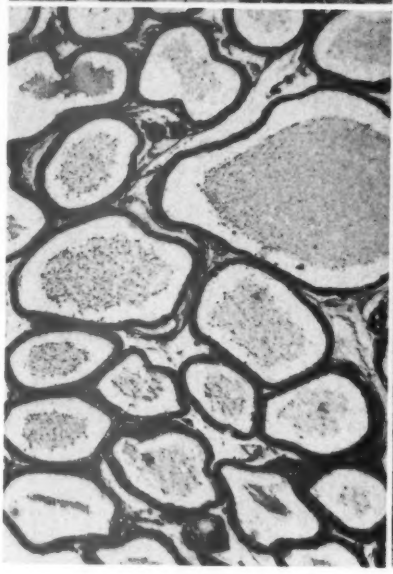

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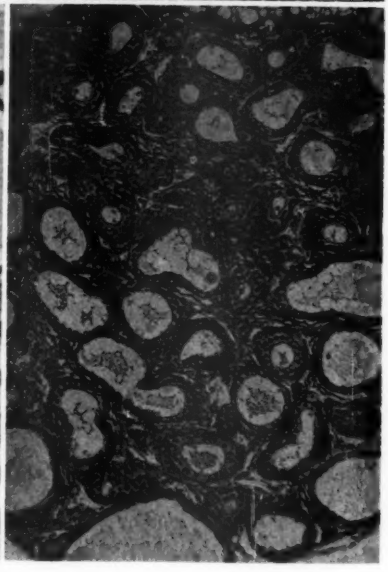

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FIG. 14. Liver section of a tryptophan-deficient rat exhibiting the maximum amount of lipidosis. × 430.

FIG. 15. Frozen section of liver from a tryptophan-deficient rat, stained with osmic acid to demonstrate moderate intracellular lipidosis. × 430.

FIG. 16. Frozen section of an adrenal gland from a normal control rat, stained with Sudan black B to demonstrate the distribution of cortical lipid. × 100.

FIG. 17. Frozen section of an adrenal gland from a semistarved control rat, stained with Sudan black B to demonstrate the distribution of cortical lipid. × 100.

FIG. 18. Frozen section of an adrenal gland from a tryptophan-deficient rat, stained with Sudan black B to demonstrate reduction in fascicular width and the distribution of lipids. × 100.

FIG. 19. Frozen section of an adrenal gland from a tryptophan-deficient rat, stained with Sudan black B to demonstrate the extreme loss of cortical lipids. × 100.

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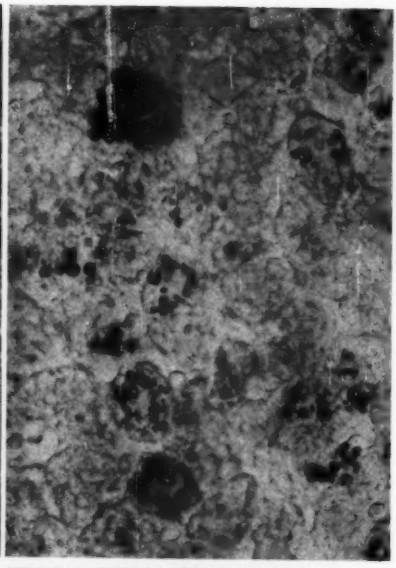

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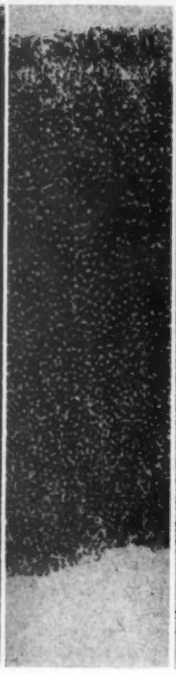

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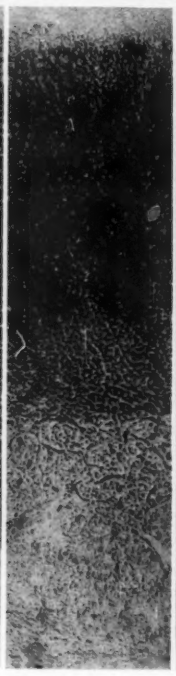

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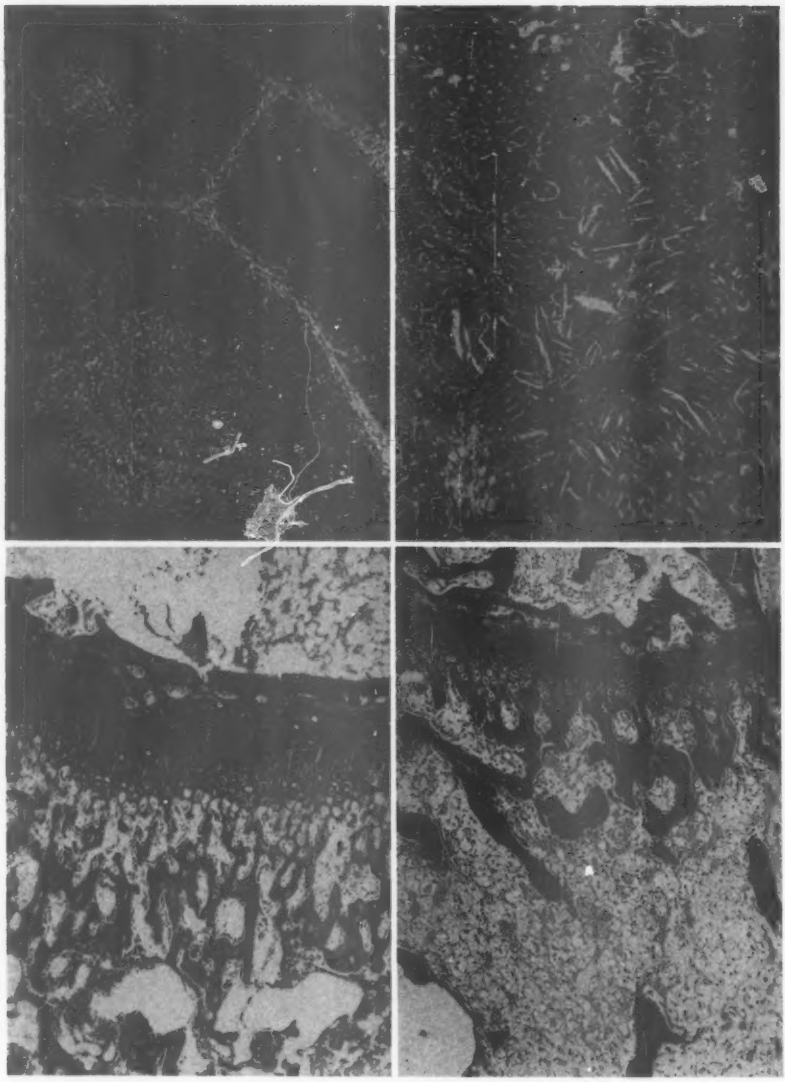
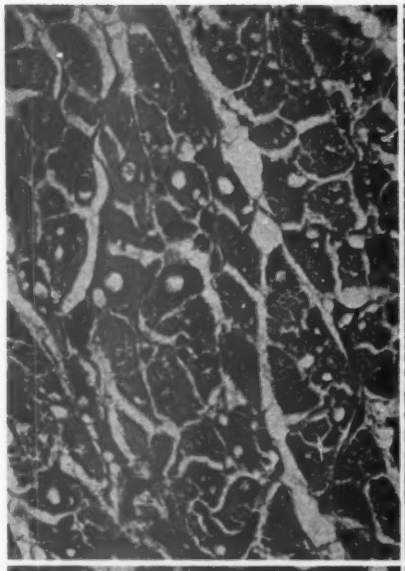


FIG. 20. Thymus from a semistarved control rat. The cortical and medullary zones are intact. × 100.

FIG. 21. Thymus from a tryptophan-deficient rat, showing intense involution, crystalline deposition, and giant cell infiltration. × 100.

FIG. 22. Tibial epiphysis of a semistarved control rat. × 50.

FIG. 23. Tibial epiphysis of a tryptophan-deficient rat. × 50.



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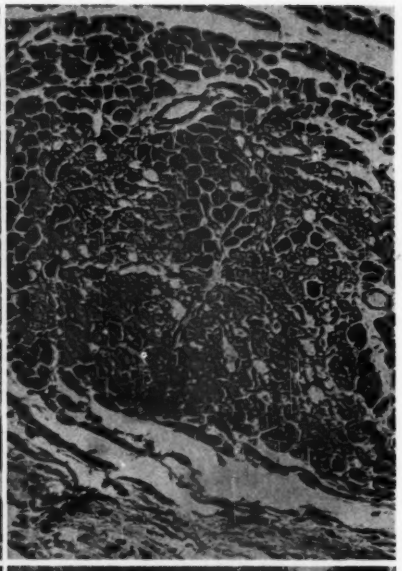

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FIG. 24. Karyolysis and cytoplasmic vacuolation in the myocardium of a tryptophan-deficient rat. × 430.

FIG. 25. Myocardial lesion in a tryptophan-deficient rat, with hyalinization of the necrotic area. × 100.

FIG. 26. Necrotized area in the myocardium of a tryptophan-deficient rat, showing early replacement of damaged muscle fibers by a loose areolar stroma. × 430.

FIG. 27. Invasion of fibroblasts into a necrotic myocardial lesion of a tryptophan-deficient rat. × 100.

LEPROSY: PATHOLOGIC CHANGES OBSERVED IN FIFTY CONSECUTIVE NECROPSIES *

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The purpose of this presentation is to record the gross and microscopic changes observed in necropsy material from leprosy patients. It consists of a review of 50 consecutive necropsies performed at the National Leprosarium (U.S. Public Health Service Hospital) at Carville, Louisiana.

Relatively few detailed studies have been reported in the English literature of the necropsy findings in leprosy. One of the earliest, that of Hansen and Looft translated into English by Walker[1] in 1895, remains among the best. Their study represents a compilation of 20 years' work following the discovery of the bacillus *Mycobacterium leprae* by Hansen in 1874, and includes a discussion of necropsy findings in 125 cases. Although some of their conclusions are no longer tenable, remarkably little improvement can be made on their original descriptions after 60 years.

An early article by Mitsuda (1902) discussing lepra cells and their distribution in certain organs was translated into English in 1936.[2] In 1937, Mitsuda and Ogawa[3] briefly discussed the gross findings in 150 necropsies from the Aiseien Leprosarium in Japan.

Black,[4] in 1938, reported a number of observations from 75 necropsies performed by him at the National Leprosarium. Kean and Childress,[5] in 1942, summarized the findings in 103 necropsies performed by several pathologists in Panama. The detailed reviews by Fite,[6,7] in 1941 and 1943, are based in part on necropsy material, and include an extensive review of the literature on tissue changes in leprosy in general. Numerous excellent illustrations of necropsy lesions are included in the *Atlas of Leprosy* by Mitsuda[8] published in 1952.

MATERIALS AND METHODS

All of the 50 cases presented in this study concerned patients at the National Leprosarium where 46 of the gross necropsies were performed. The remaining 4 gross necropsies were performed at the New

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Orleans U.S. Public Health Service Hospital, where the tissues from all 50 cases were studied microscopically and the clinical data analyzed.

Routine sections were made of heart, lung, spleen, liver, adrenal gland, kidney, testis, epididymis, skin, peripheral nerves (usually ulnar), gastrointestinal tract, lymph node, thyroid gland, pituitary body, bone, bone marrow, eye, brain, and spinal cord. Tissues were removed from additional sites when indicated. All of the tissues were fixed in 10 per cent formalin. Routine hematoxylin and eosin stains, as well as acid-fast stains, were made of all tissues. The acid-fast quality of *Myco. leprae* is more difficult to demonstrate than that of *Mycobacterium tuberculosis*, and in our experience the Fite-Cambre-Turner[9] technique is superior to other modifications of the Ziehl-Neelsen stain. Bennhold's congo red and the crystal violet stains gave the most satisfactory results for demonstration of amyloid. Mallory's trichrome stain was useful in evaluating late testicular changes.

Analysis of Cases

During the 5½ years covered by this study (September, 1948, through April, 1954), the average census at the National Leprosarium was 385 patients. Seven per cent of these were considered to have leprosy of the tuberculoid type and the majority of the remaining were considered to be of lepromatous type. Of the 50 cases in this series, 48 were of lepromatous type and 2 were of tuberculoid type. Therefore, this review is essentially one of lepromatous leprosy. In addition to being the predominant type at the National Leprosarium, it is also the type most frequently seen in the United States.[10] The 50 cases represent 92.6 per cent of all the deaths that occurred at the National Leprosarium during the 5½-year period of this study.

Thirty-four of the patients in this series were men and 16 were women, giving a 2:1 ratio which is approximately the figure given for the sex distribution of leprosy throughout the world.[11] The race was listed as white in 29 cases, Mexican in 10, colored in 8, and Chinese, Japanese, and Filipino, respectively, in the 3 remaining cases.

The average age at the time of death was 58.8 years, the youngest patient being 31 and the oldest 79 years. The average length of life from the onset of obvious signs and symptoms of leprosy was 20 years. Five of the patients gave a family history of leprosy in one or more relatives.

Sister Hilary Ross who performed the serologic tests for syphilis on these patients has previously reported[12] the high incidence of false positives on patients with leprosy. At least one, and in most cases many different, standard tests for syphilis were performed on all but

one patient in this series. Thirty-four of these patients had at least one positive serologic test, giving an incidence of 68 per cent. In 3 patients a doubtful reaction and in 12 a negative reaction was obtained. Despite this, in no case at necropsy could unequivocal evidence of syphilitic changes be found either grossly or microscopically.

Mycobacterium leprae was found in at least one tissue in 34 of the cases. The tissues in which it was possible to demonstrate these organisms were peripheral nerves (usually ulnar), skin, testis, epididymis, liver, eye, spleen, lymph node, bone, bone marrow, adrenal gland, nasal mucosa, kidney, pharynx, and larynx. In 12 of the cases, *Myco. leprae* was seen within visceral organs such as the spleen, liver, and adrenal gland. In an additional 3 cases old miliary lepromas were seen in the viscera, although no bacilli could be demonstrated. If lepra bacilli were found in the parenchymatous organs, they usually were present also in the skin and peripheral nerves.

Myco. leprae was still clinically demonstrable in skin scrapings up to the time of death in 34 of the patients. Ten of the patients had 12 consecutive negative skin scrapings (clinically arrested), and 6 had several consecutive negative skin scrapings immediately prior to death. In 26 of the cases with positive skin scrapings prior to death, organisms were demonstrated in necropsy tissues available. Of the 10 patients who were considered to be clinically arrested (12 consecutive negative skin scrapings), *Myco. leprae* was demonstrated in the tissues of 6. In the 6 additional cases with several consecutive negative skin scrapings prior to death, organisms were present in the tissues of 2 patients.

Thirty of the 50 patients had received sulfone drug therapy for 2 years or more. In 22 of these cases lepra bacilli were demonstrable in necropsy tissues.

Changes of secondary amyloidosis were seen in 23 of the cases in one or more tissues, the organ most frequently involved being the kidney. Other sites included the spleen, liver, adrenal glands, lymph nodes, stomach and rectal mucosa, and arteriolar walls of the thyroid gland, pituitary body, and pancreas. The sites of amyloid change in the parenchymatous organs such as spleen, liver, adrenal glands, and kidney were unrelated to the presence or absence of bacilli in these organs. No amyloid was found in the bone marrow in the 32 cases in which sections were taken.

When the kidney was involved by amyloid change it was usually quite markedly altered. As a result, in 19 of the cases (38 per cent), the mechanism of death was one of renal insufficiency with terminal uremia, bronchopneumonia, and/or pulmonary edema. In 9 of the

cases a malignant neoplasm was the cause of death. In 7 cases active pulmonary tuberculosis was the primary cause of death. In the remaining 15 cases various diseases such as hemorrhagic pancreatitis, myocardial infarction, and cerebral hemorrhage were responsible for the patient's demise. In only one case in the entire series (A-1950), a patient with active tuberculosis, was active widespread lepromatous leprosy believed to be a major contributing factor in the mechanism of death.

GROSS PATHOLOGIC FINDINGS

In the following sections describing the gross and microscopic findings, many of the observations are of necessity composite. Upon inspection of the body, the disfigurement of the nose, eyes, extremities, and skin was quite obvious. Corneal opacities were frequent and in some cases the eyes had been enucleated. "Saddle" nose deformity of varying degrees usually was present. The ear lobes were often enlarged and redundant. The eyebrows and eyelashes were sparse, especially laterally, and some patients had none at all. Considerable induration of the facial skin and underlying tissues resulted in the typical leonine facies of leprosy.

The skin lesions varied considerably, depending upon the activity of the disease at the time of death. Areas of irregular pigmentation, old scars from burns or lepromatous nodules, and a diffuse atrophy of skin over wide areas of the body, with thin "onion skin" wrinkling, were seen. Hypopigmented areas were frequent in the skin of deeply pigmented persons. A few patients presented raised, sometimes erythematous areas of apparently active lesions.

Obvious deformities of the hands (*"main-en-griffe"*) and feet often were present with resorption of bone and shortening of fingers, toes, and sometimes other bones. Usually the shortened finger or toe had a small, distorted nail remaining at its tip, for the digits in fact seldom "fall off" as in the cicatrizing disease ainhum, but rather undergo a progressive resorption from the tip. Many patients had trophic ulcers of the extremities and some had had previous amputations. Muscle atrophy, especially of the interossei of the hands, was prominent in many cases with marked nerve involvement. Usually the ulnar nerves were palpably enlarged. Testicular atrophy was usually quite marked. Several cases presented gynecomastia.

Review of Organ Systems

Respiratory System. The lesions of leprosy seen in the respiratory system of these 50 patients were confined entirely to the upper portion

of the tract. In the 3 cases in which the nasal mucous membrane was examined the characteristic picture was a diffuse infiltration by chronic inflammatory cells, chiefly lymphocytes and many vacuolated histiocytes ("lepra" or Virchow cells) with occasional globi. Globi are rounded, compact masses of lepra bacilli in the center of vacuoles or spaces that measure up to 100 μ in diameter. Globi are situated within giant cells or sometimes within macrophages having only a single nucleus.[13,14] Sometimes, the vacuole becomes so large the nucleus of the cell is hard to see or is not present at all.

In the more advanced cases, numerous acid-fast bacilli were seen within the lepra cells and globi, as well as diffusely throughout the intercellular spaces and overlying epithelium. Ulceration and perforation of the nasal septum were common, with destruction of nasal cartilage and bone resulting in varying degrees of "saddle" deformity. In a few of the more severe cases the nasopharynx, oropharynx, peritonsillar tissues, and larynx were involved by diffuse lepromatous infiltrate similar to that described in the nasal septum.

The respiratory tract below the larynx was not involved in any of the 50 cases. Three of our cases presented lipid granulomas of the lung parenchyma which were attributed to the use of chaulmoogra oil nasal spray or nose drops over a period of many years. In another case (A-2005) lesions of classical Boeck's sarcoid were seen in the lungs as well as in intrathoracic and retroperitoneal lymph nodes. No relationship could be ascertained between the sarcoid lesions and the patient's leprosy, which did not involve the lymph nodes examined.

Cardiovascular System. Direct involvement of the heart, aorta, or other great vessels was not seen. In one case (A-1723) a few *Myco. leprae* were seen within the media of the aorta adjacent to a large lymphosarcomatous mass in which there were large numbers of organisms. Involvement of blood vessel walls of the skin, nerves, and testes was very frequent and was uniformly present in the more active lesions.

Spleen. The weight of the spleen varied from 40 to 520 gm., and averaged 240 gm. Externally, the spleen was not remarkable except for its size and for a rare plaque of white, glistening, fibrous tissue or adhesions. Only rarely were miliary lepromas or amyloid deposits large enough to be visible grossly and therefore distinguishable from malpighian corpuscles.

Microscopically, 8 of the 49 spleens sectioned contained miliary lepromas. These were composed of vacuolated histiocytes, lymphocytes, and occasional globi and were located in every part of the

parenchyma in both the red and white pulp, often around blood vessels (Fig. 1). In 6 of the 8 cases with lepromas, acid-fast organisms were demonstrable.

Amyloid was seen in 17 of the spleens sectioned. It was located chiefly within the thickened walls of arteries of medium and small size as well as in distinct masses in the centers of malpighian corpuscles and elsewhere in the pulp (Fig. 1).

Liver. The weight of the liver varied from 890 to 2,550 gm., and averaged 1,604 gm. As in the spleen, the lepromas usually were too small to be seen grossly. In those cases with marked amyloid change the liver was usually enlarged, and the cut surface presented irregular tan areas throughout (Fig. 3).

Miliary lepromas were seen microscopically in 12 of the 50 livers sectioned and in 10 of the lepromas organisms were demonstrable. The lepromas often were located around portal triads and central veins, but were seen also along the sinusoids, apparently arising in Kupffer cells as well (Fig. 2). The gallbladder and bile ducts were never seen to be involved, although lymph nodes around the duodenum and major biliary ducts sometimes contained lepromas with organisms. Replacement of liver cord cells by amyloid was seen in 17 of the cases (Fig. 2).

Gastrointestinal Tract. The esophagus, stomach, and small and large intestines were free of involvement by lesions due to leprosy per se. In 3 cases, however, amyloid change was prominent in the mucosa and submucosa of the stomach and in one case the rectal mucosa showed similar amyloid change. The pancreas was not involved in any of the cases examined. Occasionally, in a case with widespread amyloid disease, the pancreatic vessel walls shared in the change.

Urinary Tract. The weight of the kidneys varied from 75 to 300 gm., and averaged 148 gm. The predominant lesion seen in 19 of the 50 cases was a varying degree of usually quite severe amyloidosis. The corticomedullary junctions often were indistinct and the kidneys pale, especially the cortical portions (Fig. 4). In a number of cases nephrosclerotic changes were associated with those due to amyloid, but the latter were usually predominant. Microscopically, the amyloid was seen beneath the basement membranes of the glomerular tufts and convoluted tubules and also within the walls of many smaller arteries and arterioles. Many glomeruli were completely replaced by amyloid (Fig. 5). Protein casts were seen in some kidneys.

In 2 cases a few small collections of vacuolated histiocytes containing *Myco. leprae* were seen within glomerular tufts. The ureters, bladder, prostate, and urethra were not involved by lepromatous changes in any case.

Reproductive Organs. Lesions of the female genital tract were not seen in any of the 16 women. In the male, however, the testes were seldom free of lesions and usually were quite small.[15] In 24 of the 33 cases in which sections of a testis were available, there was marked atrophy, with moderate to minimal atrophy in all but one of the remaining cases. The picture varied from a very active lepromatous infiltrate of lymphocytes, vacuolated histiocytes, and globi within and primarily between seminiferous tubules, to one of increasing degrees of hyalinization and thickening of the basement membranes and eventual complete fibrous replacement of all tubules (Fig. 6). Blood vessel involvement by an active lepromatous lesion frequently was seen as an early lesion, especially near the tunicae of the testes (Fig. 7). *Myco. leprae* was found in 16 of the 33 testes examined. Islands of interstitial cells were prominent in many of the advanced cases and in a few there actually appeared to be hyperplasia of these cells. In many cases the epididymides were involved by a lepromatous infiltrate similar to that seen in the adjacent testicular body.

Endocrine Glands. As in the liver and spleen, adrenal lesions consisted of miliary lepromas seen most frequently near the junction of the cortex with the medulla and within the medulla. Acid-fast organisms within lepromas were found in 5 of the 46 cases in which adrenal sections were available. In 16 of the adrenal glands sectioned, amyloid change was noted, principally between and replacing cords of cells in all three layers of the cortex (Fig. 8).

Few changes were observed in the thyroid or pituitary glands except for occasional deposition of amyloid within the walls of small arterioles. In a few cases with marked testicular atrophy, eosinophilic cells in the anterior lobe of the pituitary body were quite prominent. Clear cell hyperplasia of the parathyroid glands was seen in some cases with marked renal amyloidosis.

Hematopoietic System. The bone marrow shared in the widespread miliary lesions in some of the advanced cases. In 6 of the 32 bone marrows sectioned, *Myco. leprae* was demonstrable within lepromas (Fig. 9). No specific clinical hematologic pattern was seen; however, a severe hypochromic microcytic anemia was present in several cases. Several of the more active cases with widespread miliary lesions had very hyperplastic bone marrow. Amyloid was not seen in the marrow.

Lymphatic System. The lymph nodes draining skin areas involved by lepromatous infiltrate and often those in the thoracic and abdominal cavities were enlarged and contained collections of vacuolated histiocytes (Fig. 10). *Myco. leprae* was found in the lymph nodes in 7 of the 25 cases in which sections were made. One lymph node contained

large deposits of amyloid as well. The peritonsillar tissues were involved by lepromatous infiltrate in one patient who had many active lesions from the nose to the larynx.

Skin. The gross changes of the skin have been discussed. In 21 of the 44 cases with skin sections available, *Myco. leprae* was demonstrated in characteristic lepromatous infiltrates of the dermis. Usually a "free zone" of varying width was seen between the epidermis and the infiltrate (Fig. 11). The infiltrate consisted of coalescing aggregates of vacuolated histiocytes, lymphocytes, and occasional globi characteristically situated around blood vessels and nerves, and occasionally around adnexal structures (Fig. 12). Sweat glands and other adnexal structures in old lesions often were atrophic or replaced completely by the inflammatory reaction. Organisms were found within the vacuoles of histiocytes, within globi, and within adnexal structures, as well as lying free in the dermis and occasionally in the epidermis. They were seen frequently within the walls of small blood vessels and in several cases within the walls of larger vessels in the dermis.

Many cases exhibited only varying degrees of thinning of the epidermis and atrophy of dermal structures with a scattering of residual chronic inflammatory cells. The skin sections from the 2 cases listed as of tuberculoid type fell into this category with only residual atrophic changes.

Central Nervous System. The brain was examined in 37 and the spinal cord in 13 cases. Unequivocal involvement of the central nervous system by *Myco. leprae* was not found.

Peripheral Nerves. The ulnar and superficial peroneal nerves were enlarged in most cases as were other nerves in some. Many nerves contained small lepromatous lesions while in others the infiltration was quite extensive and largely obliterated the normal architecture (Fig. 13). *Myco. leprae* was demonstrable in 25 of the 47 cases in which peripheral nerve sections were made. Organisms were found within vacuolated histiocytes and globi, within axis cylinders, and within blood vessel walls both within and around the nerve trunks (Fig. 13). In almost every case, including the 2 tuberculoid cases, there was varying but usually quite extensive, fibrous thickening of the endoneurium, perineurium, and epineurium. In the nerves with the more extensive fibrous tissue replacement, few or no organisms were seen. Calcification of the ulnar nerve with extensive fibrosis was found in one patient (A-2113) who had had lepromatous leprosy for over 60 years (Fig. 14).

Eyes. The principal lesions in the eyes of the lepromatous cases were

iridocyclitis and keratitis, both superficial and deep. Diffuse lepromatous infiltration of the ciliary body, iris, and choroid was seen and *Myco. leprae* was demonstrated in many of these cases (Fig. 15). The cornea and periorbital tissues also shared in the lepromatous infiltrate in some cases. Occasionally the ocular lesions appeared quite active when the rest of the tissues of the body contained only residual changes which were apparently inactive or healed. In no case were there demonstrable lesions in the posterior half of the eye or in the optic nerve.

Musculoskeletal System. In several cases in which the calf muscles were examined there was extensive yellowish fatty replacement of large bundles of muscle fibers. The circumference of the calf and the bulk of the muscles remained normal in these cases. Microscopically, the sarcolemma remained intact, but the sarcoplasm had lost its striations and was partially or entirely replaced by fat (Fig. 16).

Characteristically, there was resorption of the bones of the phalanges and in some cases resorption of more proximal bones. Grossly, these bones were cut easily without decalcification. Microscopically, varying areas of bone resorption and new bone or osteoid formation were seen occurring simultaneously. These were associated with chronic inflammatory cellular infiltration and extensive fibrous tissue reaction. Lepromatous infiltrates within the fatty marrow spaces and within the fibrous tissue in and around the periosteum contained acid-fast organisms.

Discussion

It must be remembered that the foregoing presents the pattern of lepromatous leprosy as seen in necropsies at the National Leprosarium in the United States and that the pattern of leprosy in many other parts of the world is quite different. It should be recalled, also, that in the natural history of the disease the tendency is toward spontaneous remission after many years. The so-called "burned out" cases may reveal few or no organisms and are left only with the residual neural and other tissue damage as described in many of the present cases. One of the oldest patients in this series became blind from leprous changes in 1898, 8 years after the clinical onset of his leprosy. He refused virtually all specific therapy except for sporadic doses of chaulmoogra oil totalling approximately 1,000 cc. Several years prior to death, over 60 years after the onset of his leprosy, skin scrapings were positive only occasionally. No organisms were demonstrable at necropsy.

It should be pointed out that 30 of these patients (60 per cent) re-

ceived sulfone therapy for at least 2 years, and 10 patients received sulfone therapy for a shorter period. It is believed that this treatment has influenced to some extent the pattern of the disease in some of these patients. Undeniably beneficial effects are produced clinically by the sulfones, as reported recently by Chang, Wolcott, and Doull[16]; however, they pointed out that bacteriologic improvement may lag behind clinical improvement for years. Skin scrapings were positive clinically just prior to death in 23 of the 30 patients who received sulfone therapy for at least 2 years. As mentioned before, *Myco. leprae* was demonstrable also in necropsy tissues in 22 of these cases. Moreover, it is believed that if additional multiple sections of skin and nerve had been taken, organisms would have been found in a higher percentage of cases.

As seen by the average duration of life of 20 years after the recorded onset of obvious signs and symptoms, leprosy per se is not a rapidly fatal disease. Also, the average age at the time of death of just under 59 years is less than 10 years below that of the population as a whole. As mentioned, in only one patient in this series was widespread leprosy itself considered to be a major factor in the immediate cause of death. However, the disabling features of leprosy often were seen, as in the contractures, resorbed digits, neurotrophic ulcers, renal insufficiency, and blindness.

While leprosy was not an immediate cause of death, it very frequently produced secondary changes which in turn were responsible eventually for the patient's demise. Thus in 38 per cent of the cases, amyloidosis of the kidney, secondary to the leprous infection, produced renal insufficiency with uremia, often coma, bronchopneumonia and/or pulmonary edema, and death. The cause of death in another 14 per cent was active pulmonary tuberculosis to which the patient might have been predisposed by the debilitating effects of the leprous infection. One might assume, then, that in approximately 50 per cent of the cases, leprosy was indirectly responsible for death. In the other 50 per cent, diseases to which any person might succumb, such as neoplasms and myocardial infarction, were the causes of death.

From the foregoing descriptions, it can be seen that few tissues in the body were free from demonstrable involvement by lepromatous leprosy at necropsy. The principal ones not involved included the lower respiratory tract, the heart and great vessels, the gastrointestinal tract, the central nervous system, and the female reproductive organs. Isolated instances of involvement of most of these sites have been reported in the literature,[2,7] but their occurrence must be exceedingly uncom-

mon. Viscera such as spleen, liver, and adrenal gland contained lepromatous lesions in one third of the cases in this series. In 6 of the 10 patients believed to be clinically arrested (12 consecutive negative skin scrapings), organisms were demonstrated at necropsy.

A striking feature secondary to leprosy in these patients was the frequency with which amyloid was seen (almost one half of the cases). When the kidney was involved by amyloidosis, it usually was very markedly altered and resulted, as mentioned, in marked renal insufficiency which was incompatible with life. The pathogenesis of the deposition of amyloid, while obviously related to the leprous infection of the body in general, still remains theoretical.[17]

Summary

The pathologic changes of leprosy as seen in the United States are presented by reviewing in detail 50 consecutive necropsies from the National Leprosarium (U.S. Public Health Service Hospital) at Carville, Louisiana.

Two of the cases were considered to be of the tuberculoid type and 48 of the lepromatous type of leprosy, the predominant type at the National Leprosarium as well as in the United States in general.

Mycobacterium leprae was demonstrable in at least one tissue in 34 of the cases (68 per cent). These tissues included peripheral nerves (usually ulnar), skin, testis and epididymis, liver, eye, spleen, lymph node, bone, bone marrow, adrenal gland, nasal mucosa, kidney, pharynx, and larynx. Organs such as spleen, liver, and adrenal gland contained lepromatous lesions in one third of the cases.

The average duration of life after the onset of obvious signs and symptoms was 20 years and the average age at the time of death was just under 59 years. While leprosy is seldom a rapidly fatal disease, over a period of many years it often results in the marked debilitating effects of contractures, neurotrophic ulcers, renal insufficiency, and blindness.

Secondary amyloid changes were seen in 23 cases in one or more tissues, the organ most frequently involved being the kidney. Renal insufficiency secondary to the amyloid change was the most frequent cause of death in the series (38 per cent).

We wish to acknowledge the help and cooperation of the entire Staff of the U. S. Public Health Service Hospital, Carville, Louisiana; in particular, Dr. Rolla R. Wolcott, for help in analysis of the clinical data and Sister Hilary Ross for the gross photographs. We are indebted also to Dr. Chapman H. Binford of the Armed Forces Institute of Pathology for the photomicrograph of the calcified nerve (Fig. 14) and for the critical appraisal of the manuscript.

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LEGENDS FOR FIGURES

FIG. 1. Spleen showing deposition of amyloid material within and around a malpighian corpuscle as well as clusters of vacuolated histiocytes (miliary lepromas) below. Hematoxylin and eosin stain. × 228.

FIG. 2. Liver. Centrally, there is a miliary leproma surrounded by large areas of replacement of liver cords by pale-staining amyloid material. Small, darker cells are remaining liver cord cells. Hematoxylin and eosin stain. × 300.

FIG. 3. Cut section of liver showing widespread, diffuse, pale tan areas of amyloid.

FIG. 4. Cut section of kidney showing loss of normal markings with large, pale areas of amyloid in both cortex and medulla.

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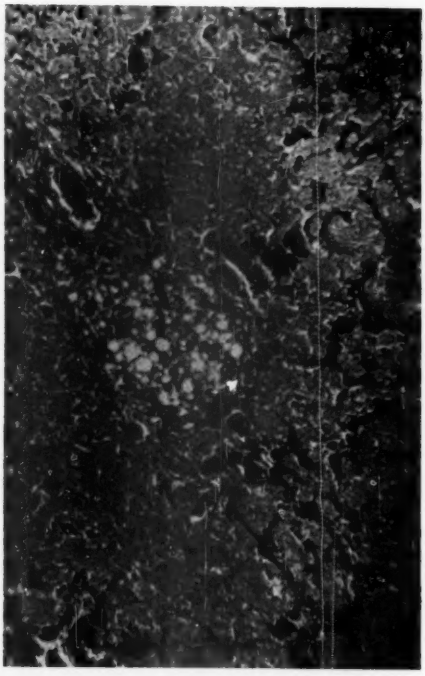

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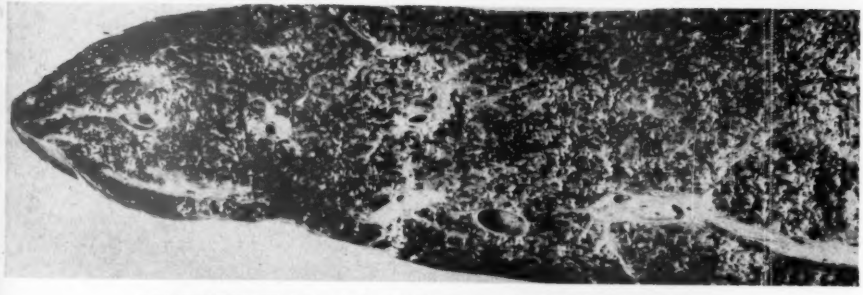

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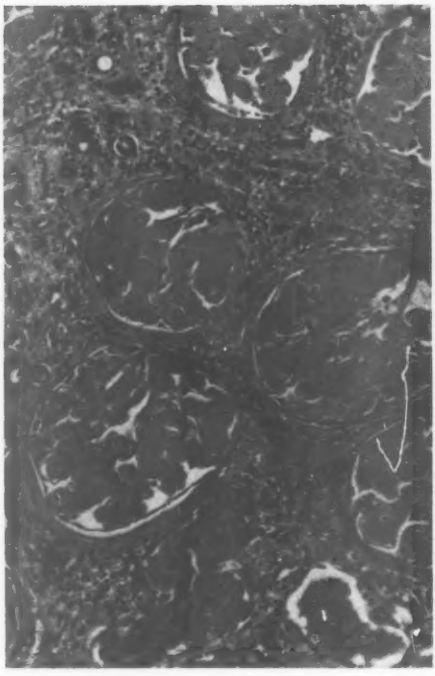


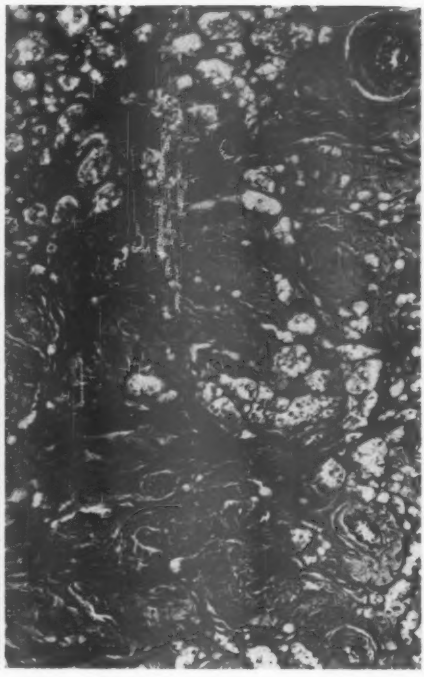


FIG. 5. Kidney with enlarged glomerular tufts showing marked replacement by amyloid. Extensive loss of intervening tubules may be noted also. Hematoxylin and eosin stain. × 228.

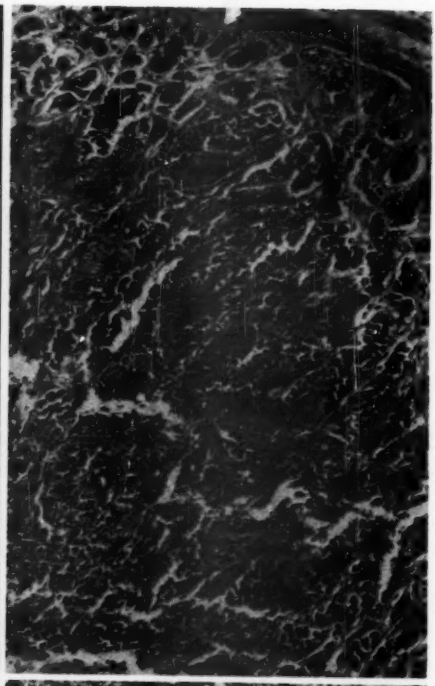
FIG. 6. Testis. No normal seminiferous tubules can be seen. In the central area some can be seen completely replaced by dense, hyalinized, fibrous tissue. Marked thickening of blood vessel walls is seen at the right. The pale, vacuolated areas consist entirely of diffuse lepromatous infiltrate. Hematoxylin and eosin stain. × 228.

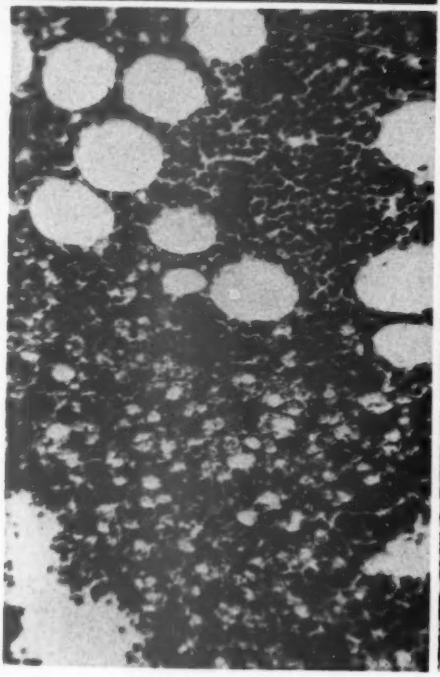
FIG. 7. Oil-immersion photomicrograph of a testicular blood vessel showing marked infiltration of *Mycobacterium leprae* bacilli within the enlarged endothelial cells and within cells and globi deeper in the arterial wall. Acid-fast stain. × 900.

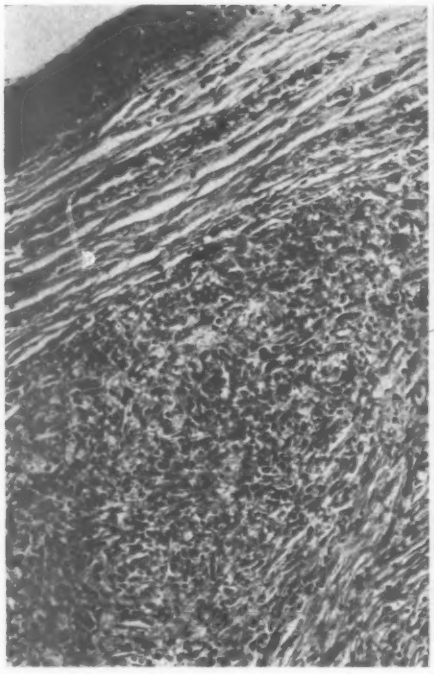
FIG. 8. Adrenal gland with extensive replacement of cortical tissue by pale-staining amyloid. Residual dark cortical cells can be seen beneath the capsule. Hematoxylin and eosin stain. × 228.

FIG. 9. Lumbar vertebral bone marrow showing normal hematopoietic tissue above and lepromatous infiltrate below. Of note are the normal appearing, large fat cells above, which are in contrast to the smaller, numerous, vacuolated histiocytes below. Hematoxylin and eosin stain. × 524.

FIG. 10. Lymph node with large area of lepromatous infiltrate (vacuolated histiocytes) below and small deposits of amyloid above (some within the thickened walls of arterioles). The lymph node capsule is seen in the upper right corner. Hematoxylin and eosin stain. × 228.







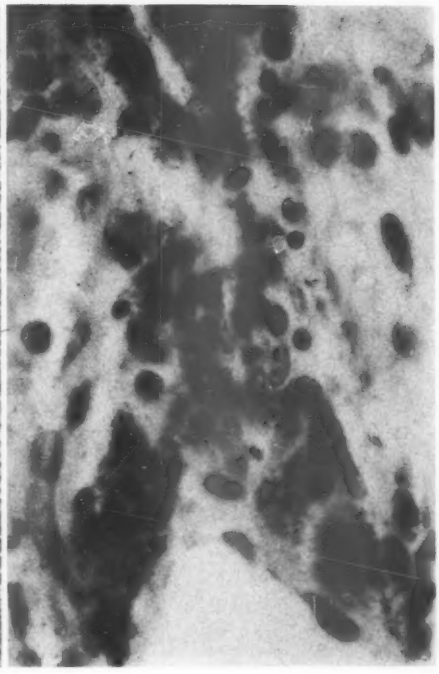


FIG. 11. Skin showing characteristic "free zone" between epithelium and lepromatous infiltrate in the dermis. Coalescing aggregates of lymphocytes and macrophages make up the bulk of the infiltrate. The vacuoles in the macrophages in this case are quite small. There is some blunting and loss of rete pegs of the epithelium but little atrophy. Hematoxylin and eosin stain. × 228.

FIG. 12. Oil-immersion photomicrograph of skin showing large number of *Myco. leprae* within the vacuoles of macrophages in the lepromatous infiltrate in the dermis. A lymphatic space is seen below. Acid-fast stain. × 958.

FIG. 13. Longitudinal section of ulnar nerve showing lepromatous infiltrate with numerous large globi, some of which contain masses of dark-staining acid-fast bacilli. Acid-fast stain. × 524.

FIG. 14. Transverse section of ulnar nerve with extensive fibrosis and a large area of calcification. Most of the calcium has fallen out during processing; however, fragments can be seen within the defect. Hematoxylin and eosin stain. × 55.

FIG. 15. Oil-immersion photomicrograph of ciliary body of eye showing pigmented layer, upper right. Below numerous *Myco. leprae* can be seen within the lepromatous infiltrate and within a large globus in a giant cell. Acid-fast stain. × 700.

FIG. 16. Gastrocnemius muscle showing replacement of sarcoplasm and distortion of sarcolemma by fat. Hematoxylin and eosin stain. × 300.

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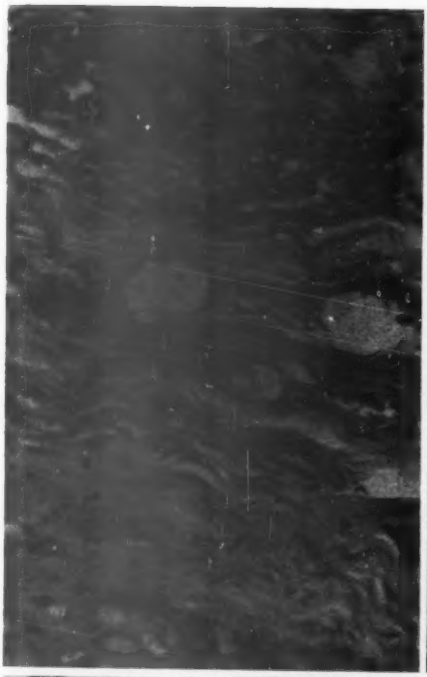

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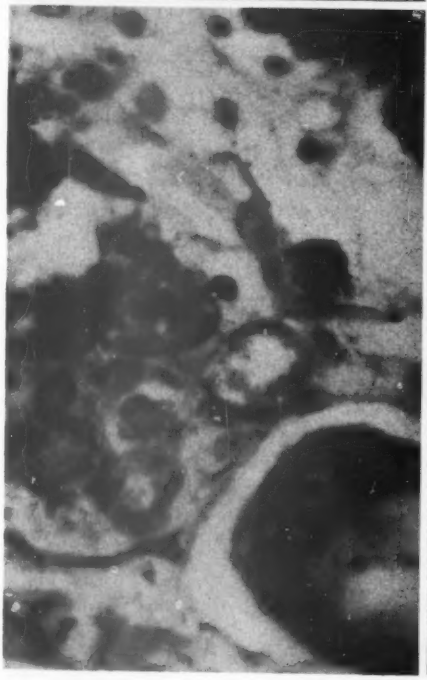

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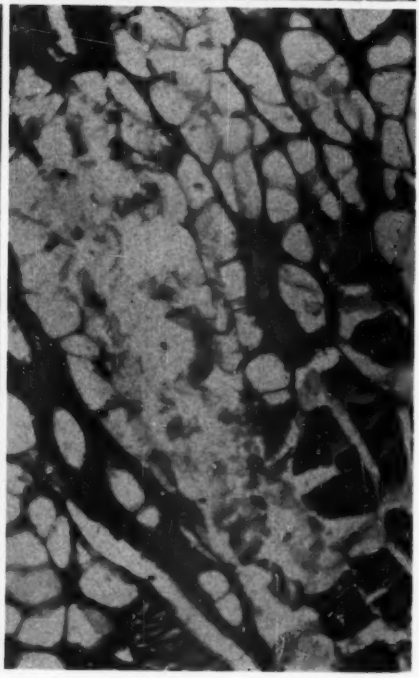

16

CYTOLOGY OF ISLET CELLS IN ALLOXAN DIABETIC RABBITS *

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Most authors agree that after the injection of alloxan and the consequent destruction and disappearance of beta cells, the pancreatic islets contain numerous non-granular islet cells.[1-4] These have been interpreted as agranular beta cells or agranular cells of an unspecified type. In contrast to our knowledge of the fate of the alpha and beta cells in alloxan diabetes, very little is known about that of the delta cell.[5,6] Aside from the description of Conn and Hinerman[7] in a case of hypoglycemia treated with alloxan in a patient suffering from an islet cell tumor, and the brief discussions by Cavallero[8] and Verne,[9] no specific mention of the delta cell in alloxan-treated animals or man has been found.

Some workers have studied the Golgi apparatus and/or the mitochondria of islet cells in order to delineate the morphologic structure and changes related to insulin secretion.[10-13] The study of the Golgi apparatus and the mitochondria of islet cells has led other workers to postulate the presence of transitional forms between acinar and islet cells.[14,15] While the histologic techniques employed by these investigators gave a positive impregnation of the Golgi apparatus, they did not permit an accurate identification of the various types of islet cells.

The origin of new islet cells of each type and the question of their interconvertibility in alloxan diabetes are subjects which remain unsettled. Some workers have reported on the formation of new alpha and beta cells from acinar cells.[13,16-18] Others have stated that the newly formed beta cells are derived from the ductular epithelium.[7,18-21] Berthoud,[18] in agreement with Ferner,[22] reported that alpha cells are transformed into beta cells, while others[8,21,23] have stated that this has never occurred in their experience.

In previous investigations on the histogenesis of the pancreatic islet of the rabbit, with methods capable of demonstrating the three types of islet cells as well as their respective cytoplasmic organelles in a single section, it was found that cells of each of these types were derived independently from duct cells and were not interconvertible.[24] In view of this finding, it appeared that a study of the changes in the

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pancreas of the rabbit after the administration of alloxan, using the same histologic methods, would clarify the following points: (1) the relationship between the delta cells, normally devoid of granules in the rabbit,[24] and the non-granular islet cells found in alloxan-treated animals; (2) the origin, if any, of new islet cells; (3) the correlation between the cytologic changes in the alpha, beta, and delta cells and the diabetic state. Although mentioned separately, these three points are found to be intimately related in a study of the dynamic morphology of islet cells.

Material and Methods

Seventy-one purebred New Zealand white rabbits were used. They weighed between 1,500 and 5,500 gm., the average being 2,500 gm. Twenty-three of these were controls. The 48 remaining received a single intravenous injection of 150 mg. of alloxan (alloxan monohydrate, Eastman Kodak Co.) per kg. of body weight, as a freshly prepared 5 per cent solution in distilled water. In establishing this group of diabetic rabbits, many were injected with alloxan, but those animals which did not develop a fasting hyperglycemia of at least 200 mg. within 48 hours after alloxan administration were discarded. Twenty-three of the diabetic animals died at various intervals and were not used for histologic study. The remaining 25 animals were sacrificed, one each at the following intervals: 15 minutes, 45 minutes, 1½ hours, 3 hours, 7 hours, 12 hours, 1½ days, 2 days, 2½ days, 3 days, 4½ days, 5½ days, 9 days, 10 days, 14 days, 28 days, 4½ months. In addition, 3 animals were sacrificed at 18 hours, 2 at 1 day, and 3 at 2½ months. Specimens from the head, body, and tail of the pancreas of each animal were processed and stained according to the techniques previously described for Masson's trichrome, Gomori's chrome alum hematoxylin, Aoyama's method for Golgi apparatus, and Schriddes' method for mitochondria. Serial sections 2.5 μ thick were studied from each block. The diabetic state was assessed by blood sugar determinations daily during the first week and twice a week from then on.

Results

The well known lesions of the pancreas in alloxan diabetes and their development were confirmed. These included selective necrosis of the beta cells[5] and glycogen infiltration of the duct cells and of the hydropic cells of the islets.[25] The detailed description that follows includes only those observations pertinent to the problems of islet cell cytology referred to in the introduction.

After the injection of alloxan and the consequent necrosis and disappearance of beta cells, the islets were composed predominantly of alpha and delta cells. The alpha and delta cells appeared to be more numerous, but while there was some evidence of proliferation of the alpha and delta cells, this was not sufficient to account for the appearance of more numerous alpha and delta cells. The latter appearance was due mainly to the concentration of the peripherally situated alpha and delta cells resulting from the destruction of the centrally located beta cells. This resulted in a reduction in size of the islets. No changes were observed in the nuclei, cytoplasm, or Golgi apparatus of the alpha cells (Fig. 1). On the other hand, the delta cells showed slight but definite variations from the normal. This, however, occurred only in animals which had persistent severe diabetes for 3 to 4 months. When stained with the Masson trichrome, the delta cell cytoplasm was sometimes paler blue than usual. Occasionally it was slightly vacuolated and in other instances the cell border became ragged. Some delta cells showed denser and more intensely basophilic nuclei (Fig. 2). The Golgi apparatus and mitochondria, however, were well within the normal range (Fig. 3).

During the first 24 hours after the injection of alloxan, the Golgi apparatus and the mitochondria of the beta cells remained intact until the cytoplasm began to disintegrate. At that time, these organelles also fragmented, and diffusely distributed black-staining granules were seen with the Schridde and the Aoyama methods. Forty-eight hours after the administration of alloxan, beta cells were exceedingly rare in the pancreas, and when present were agranular. Some were in islets, others were in relation to small ducts. In general, the Golgi apparatus of these agranular beta cells was smaller and simpler in structure than that of the controls (Figs. 4 and 5).

In mildly diabetic animals, within 1 to 2 weeks, agranular beta cells became more numerous and tended to form small, poorly vascularized islets consisting of about 20 to 40 cells when counted in serial sections. These islets usually were devoid of alpha and delta cells. The Golgi apparatus of these agranular beta cells was well developed, although usually less so than that of the controls (Figs. 5 and 6). With time, in the severely diabetic animals, the agranular beta cells first appeared swollen (Figs. 6 and 7) and later hydropic with sharp cytoplasmic borders (Figs. 2, 3, and 8). In both cases, the mitochondria became less numerous, the Golgi apparatus shrank progressively in size, and the macular zone became more prominent (Fig. 2). These changes, however, were most marked in the hydropic beta cells. Diabetic rab-

bits which were normoglycemic or nearly so at the time of necropsy, showed numerous islets with beta cells possessing typical beta granules and a Golgi apparatus similar to that of the control.

On some occasions, in severely diabetic animals, cells were found with a clear, faintly blue-tinged cytoplasm with the Masson trichrome stain, which showed a small, irregular Golgi apparatus. These cells were not readily identifiable and were thought to be either degenerated beta or delta cells.

Two to 4 hours after the injection of alloxan most animals showed mitotic activity of the duct cells, acinar cells, and the alpha, beta, and delta cells. The increased mitotic activity of the islet cells reached a maximum at 4 to 6 days and practically disappeared in about 10 days. The mitotic activity of the beta cells did not seem to result, in the majority of animals, in an increase in their numbers. This mitotic activity of the beta cells was greater in animals whose diabetes was improving.

In some animals, the ductular epithelium showed evidence of new formation of beta cells. The first indication of this process, which was usually observed within 48 hours after alloxan administration, consisted of localized hyperplastic changes involving groups of 5 to 10 cells. These cells became enlarged and syncytium-like, with more prominent nuclei (Fig. 8). After 48 hours, a few small aggregates of a new type of cell were observed, usually attached to the ductules but also in a close relationship to the acini, similar to that of the primitive beta cell in the embryo.[24] These new cells resembled somewhat, in their mode of origin and their appearance, the primitive beta cell found in the 10- to 18-day-old rabbit embryo. The primitive beta cell of the developing embryo has been identified as a cell of fixed differentiation for the beta cell series but without all the characteristics of the mature beta cell.[24] The cytoplasm of these newly formed beta cells was amphophilic with the Gomori chrome alum hematoxylin; it possessed a small juxtanuclear macular zone stained a sky blue; and its Golgi apparatus was somewhat smaller than that of a mature beta cell.

Discussion

Delta Cell Versus Agranular Beta Cell

The value of the cytologic technique used became apparent when the interrelationship of the various types of islet cells and the nongranular islet cells found in alloxan-treated rabbits was studied. These methods were particularly useful in separating most agranular beta cells (newly formed beta cells, agranular beta cells of mildly and severely diabetic rabbits, hydropic beta cells) from delta cells, which

also appeared as non-granular islet cells with the method used. The small number and the juxtanuclear location of the delta cell mitochondria, the location and character of the Golgi apparatus, the staining properties of the cytoplasm with Masson's trichrome and Gomori's hematoxylin stains are factors which, when taken together, clearly distinguish delta cells from most agranular beta cells. From my work, it appears that a large majority of the cells described as non-granular islet cells in alloxan diabetes[1-4,19,20] are delta cells. This would explain the disparity between the islet structure and the course of the diabetes in alloxan-treated animals. In many instances, agranular cells were present in such numbers that, if they were morphologically identifiable as beta cells, they would be expected to prevent a fully developed diabetic syndrome. However, since on close cytologic examination these cells proved to be delta cells, the diabetic status of the animal is accounted for morphologically.

Some workers using the Mallory-Heidenhain azan stain have reported on the presence of delta cells in alloxan diabetes in both human[7] and experimental animals.[8,9] Conn and Hinerman,[7] using a similar technique, interpreted differences in staining affinity of the delta cell as indicating damage of these cells by alloxan. Their findings are in agreement with those reported here, in that the changes in the staining characteristics of the delta cells usually were present in severely damaged islets of animals which had marked and prolonged diabetes. It is difficult, without further work, to elaborate on the significance of these changes in the delta cells.

Origin of Islet Cells and Islet Cell Interconvertibility

The results obtained in the present work tend to confirm the conclusions drawn from the study of the histogenesis of the islet, in which it was shown that the alpha, beta, and delta cells originate from the ductular epithelium, are independent cell types, and are not mutually interconvertible.[24] In the present work it was found that some new alpha, beta, and delta cells were derived by mitotic activity of preexisting ones, and some beta and delta cells were derived directly from the ductular epithelium in a manner similar to that found during the development of the pancreas.[24] Cavallero[8] similarly reported the new formation of alpha, beta, and delta cells through mitotic activity in the alloxanized rat. Contrary to the contention of Ferner[22] and in agreement with others,[21,23] I was unable to demonstrate any type of cell that could represent a transition between alpha and beta cells. Verne[9] considered the delta cells as the matrix for alpha and beta cells in alloxan diabetes. However, the methods used by him did not permit

a clear separation of the delta cell, the duct cell, and the various types of agranular beta cells as described in this work. The transformation of acinar cells into alpha or beta cells in alloxan diabetes of treated animals, as mentioned by some authors,[13,16-18] could not be confirmed.

Golgi Apparatus in Degranulated and Hydropic Beta Cell

Many problems must be solved before there can be a complete understanding of the beta cell degranulation phenomenon as it is related to other cytoplasmic organelles. Degranulation of the beta cells in normoglycemic animals receiving large amounts of carbohydrates or in diabetic animals on ad libitum diets usually has been interpreted as a sign of increased insulin secretion.[26-30] It is also generally accepted that increased secretory activity of islet cells is accompanied by increase in size of their Golgi apparatus.[10,12,26,30] The methods employed by the latter investigators, however, were somewhat incomplete. When positive impregnation of the Golgi apparatus was obtained, their preparations were not counterstained so as to identify the alpha, beta, and delta cells.[10,12] When the alpha and beta cells were identified, the Golgi apparatus was studied by its negative image only.[26,30] I[24,31] have overcome these difficulties recently by developing a staining method capable of specifically differentiating the three islet cell types while at the same time giving a positive impregnation of the Golgi apparatus. Using these methods, hypertrophy of the Golgi apparatus was never observed in the agranular beta cell of diabetic animals. Therefore, if hypertrophy of the Golgi apparatus is considered to represent hyperfunction of the beta cell, from the present experiments it can be stated with confidence that there was no hyperfunction. The hypertrophy of the Golgi apparatus observed by other authors in degranulated cells in conditions similar to ours,[26,30] but without the use of specific silver or osmic acid methods, is believed to result from the disappearance of granules which ordinarily obscure the Golgi net in sections thicker than 3 or 4 μ.

The structure of the Golgi apparatus in hydropic islet cells has not been demonstrated by the use of specific methods, to the best of my knowledge. Whether the hydropic beta cell represents a degenerating non-secreting beta cell or a hypersecreting cell cannot be decided on the basis of my experiments. Two recent observations, however, are of interest in this respect. Hydropic beta cells produced in cortisone diabetes persist in spite of the normoglycemia which follows the cessation of cortisone treatment.[32] In addition, it has been demonstrated that a segment of the pancreas infused with glucose eventually shows

extensive hydropic changes. Moreover, this segment is hyperactive as shown by the persistent hypoglycemia obtained under such conditions.[33] These last two reports would suggest that the hydropic beta cell (of the present work), in spite of its small Golgi apparatus and decreased mitochondrial population, may actually be a very active islet cell. On the other hand, Toreson[25] advanced the idea that the hydropic changes of duct and beta cells might represent a deranged regeneration of islets, and Nerenberg[34] described the embryonal alpha and beta cells as hydropic in the rat. Nerenberg, however, did not comment on their glycogen content. Whether the presence of a small Golgi apparatus in a hydropic beta cell is due to a regressive change in the beta cell or whether it is due to lack of maturation on the part of newly formed beta cells requires further investigation. It is well known that the Golgi apparatus of some cells increases in size as the cell matures or differentiates.[35]

In the present work, the agranular non-hydropic beta cells were noted to have either a normal or small Golgi net while the hydropic beta cell always had a small Golgi apparatus. One can conclude, therefore, that if some of the beta cells in alloxan diabetes were hyperactive, as one might expect, this hyperactivity was not manifested at any time by an appreciable hypertrophy of their Golgi apparatus.

Summary

A study of the pancreatic islet cells in alloxan-treated rabbits by a variety of specific cytologic methods leads to the conclusion that the large majority of non-granular islet cells present in alloxan diabetic animals are delta cells, while only a few are non-granular beta cells, and fewer still are primitive beta cells newly formed from ductule epithelium. The agranular beta cells are distinguished from delta cells by the presence in the latter of the typical Golgi apparatus and the staining quality of its cytoplasm. In no case is hypertrophy of the Golgi apparatus found in alpha, beta, or delta cells. This is so, despite the fact that the residual beta cell in the diabetic animal would be expected to be hyperfunctioning.

The present work supports the view that the alpha, beta, or delta cells are fixed cell types which are not transformed one into another. I was unable to substantiate the hypothesis that acinar cells may be transformed into beta cells either directly or indirectly via the alpha cell. It was demonstrated that new alpha, beta, and delta cells may be derived either from duct cells or by mitotic activity from pre-existing mature islet cells.

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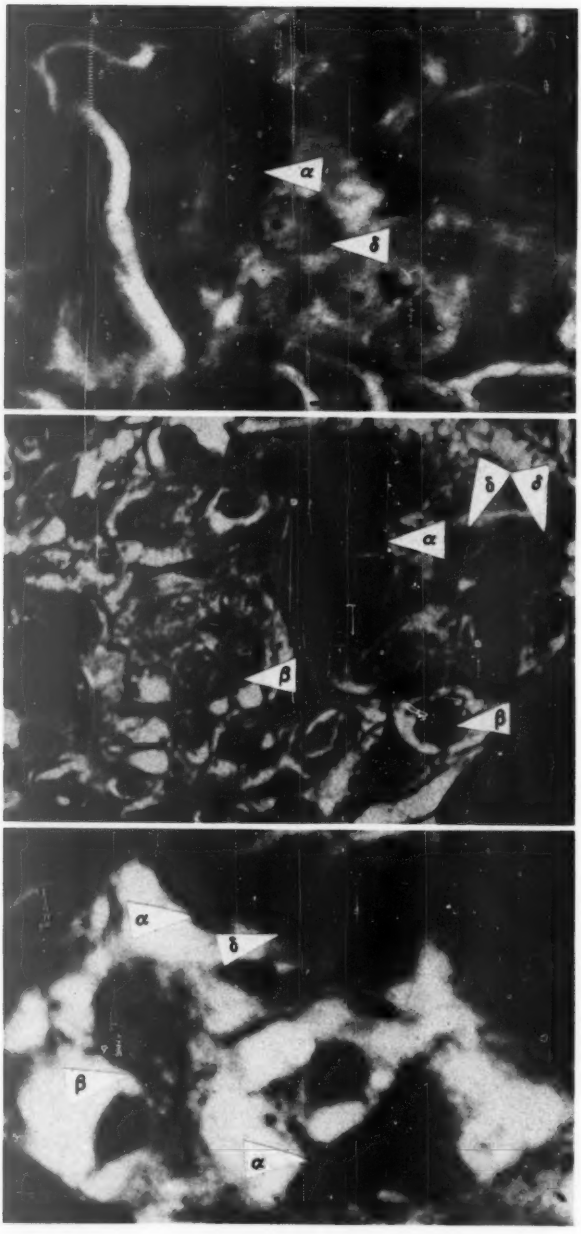
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. From a moderately diabetic rabbit of 4½ months' duration. Small pancreatic islets showing alpha and delta cells with normal Golgi apparatus. Aoyama's method for Golgi apparatus counterstained with Masson's trichrome. × 1,250.

FIG. 2. From a severely diabetic rabbit of 4½ months' duration. Pancreatic islet showing alpha cells; delta cells with small, dark nuclei and ragged cytoplasm; and hydropic beta cells with a semilunar macular zone. Aoyama's method for Golgi apparatus counterstained with Masson's trichrome. × 950.

FIG. 3. From a severely diabetic rabbit of 4½ months' duration. Pancreatic islet showing the Golgi apparatus of alpha, delta, and hydropic beta cells. Aoyama's method for Golgi apparatus counterstained with Masson's trichrome. × 1,250.


α
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1
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2
β
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α
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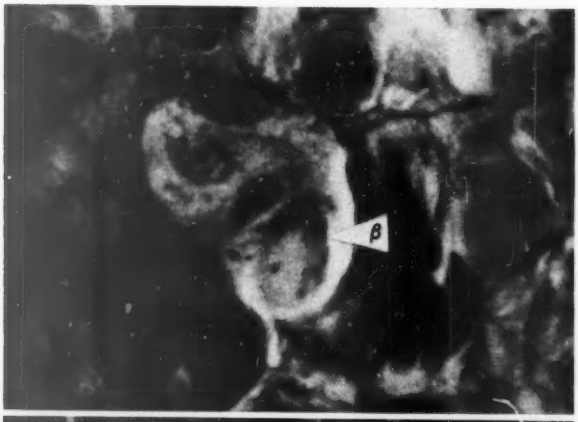
FIG. 4. From a moderately diabetic rabbit 5½ days after alloxan injection. Pancreatic islet composed of agranular beta cells with relatively small and simple Golgi net. Aoyama's method for the Golgi apparatus counterstained with Masson's trichrome. × 1,500.

FIG. 5. Pancreatic islet from a control rabbit, showing the Golgi apparatus of alpha and beta cell. Aoyama's method for the Golgi apparatus counterstained with Masson's trichrome. × 1,250.

FIG. 6. From a severely diabetic rabbit of 2½ months' duration. Pancreatic islet showing agranular swollen beta cells. The Golgi apparatus of these beta cells is smaller than that of the control (Fig. 5). Aoyama's method for the Golgi apparatus counterstained with Masson's trichrome. × 1,250.



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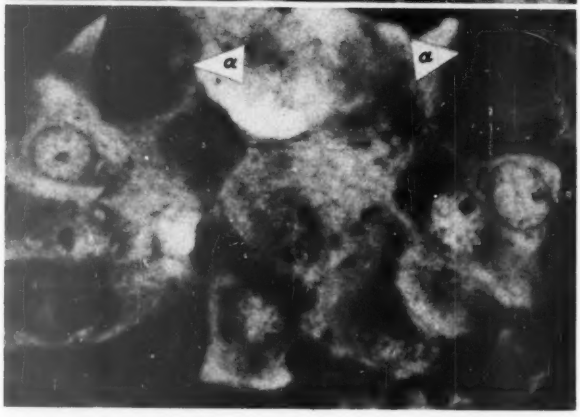

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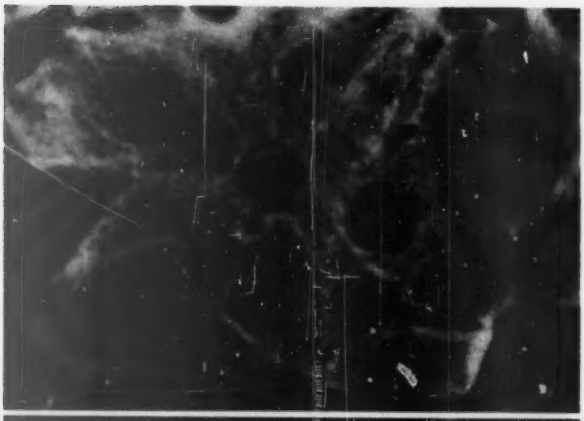
FIG. 7. From a severely diabetic rabbit of 2½ months' duration. Pancreatic islet composed of agranular beta cells. The islet is poorly vascularized and the cordonal arrangement of the beta cells has disappeared. Masson's trichrome stain. × 1,250.

FIG. 8. From a severely diabetic rabbit of 4½ months' duration. Pancreatic islet showing the small Golgi apparatus (β) and the macular area of hydropic beta cells (M). Aoyama's method for the Golgi apparatus counterstained with Masson's trichrome. × 1,250.

FIG. 9. From a moderately diabetic rabbit of 5 days' duration. Section of pancreas showing the changes in the ductular system when new beta cells are taking origin from it. These newly formed beta cells resemble primitive beta cells. The intimate relation between newly formed beta cells and acini may be observed. Masson's trichrome stain. × 750.



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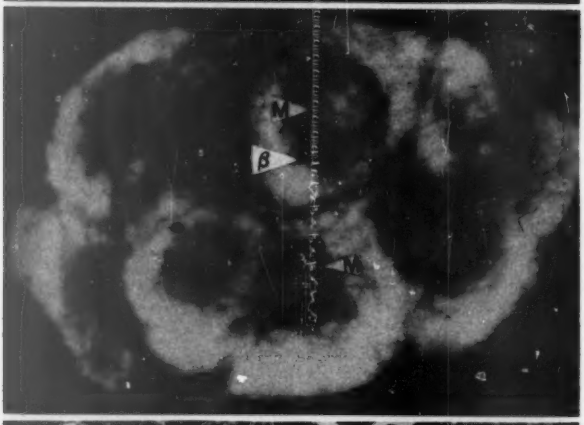

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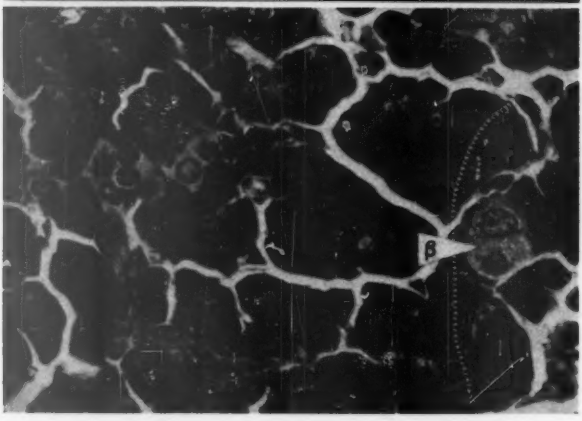

9

SUSCEPTIBILITY IN VITRO OF ADULT HORSE TISSUE TO EQUINE ABORTION VIRUS

Previously Undescribed Lesions *

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According to available information,[1-3] the agent of equine abortion produces lesions only in the aborted fetus, without signs of infection in the mare before or after parturition. The previous demonstration[4] that fetal horse tissue could be infected *in vitro* suggested that certain adult tissues should be used in this connection.

Material and Methods

Tissues. Pregnant mares were sacrificed and the fallopian tubes, portions of spleen, lung, and amnio-allantoic membrane were removed aseptically. The various tissues, including the lining of the tube, were minced separately into fragments 2 to 3 mm. in width and rinsed until clear with Hanks's salt solution.

Tissue Culture Materials. The fluid used to maintain the tissue fragments consisted of 75 per cent human ascitic fluid and 25 per cent Earle's solution; 500 units of penicillin and 100 μg. of streptomycin were added per ml. Sterility of all procedures was controlled by the usual bacteriologic techniques.

Virus. The inoculum used to initiate the present study consisted of 16th passage, fetal equine splenic tissue-culture material[4] and is referred to as a standard stored virus, the dilution being $1 \times 10^{-29.4}$. Small aliquots were kept at –30° C. The original virus preparation used to initiate the tissue culture studies[4] was prepared from the lung of a typical field case of virus abortion and all dilutions refer to this material.

Technique of Cell Maintenance. The various tissues were maintained by the classic Maitland method. For each type of tissue, eight 50 ml. rubber-stoppered Erlenmeyer flasks, containing 3 ml. of nutrient fluid, were employed, two serving as controls. Each vessel received 20 to 30 pieces of tissue. All flasks were incubated for 48 to 72 hours at 37° C. Representative test and control tissues were fixed in Zenker-

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acetic acid fluid. Initially, 0.1 ml. of standard virus was used as inoculum. The infected tissues selected for serial passage were ground to a smooth paste and diluted with like passage nutrient fluid, the amount adjusted so that in each passage the virus of the preceding passage was diluted by a factor of 1:33. Between passages the tissue culture preparations were stored at −30° C.

Complement-Fixation Methods. The original complement fixation procedure[5] for equine virus abortion, employing the 50 per cent hemolysis end-point technique, was abandoned because of the excessive amount of reagent required. In the present communication the conventional method as described by Doll *et al.*[6] was used to save materials. The various tissue antigens were ground in physiologic saline solution and stored at −30° C. The origin and preparation of the various control antigens, positive and negative sera, have been documented previously.[4,5] Essentially, the procedure utilized twofold 0.25 ml. serial dilutions of antigen, 0.25 ml. of 1:10 dilutions of serum, and 0.5 ml. of two full units of complement. Fixation was carried out overnight at 4° C. and 0.5 ml. of sensitized cells were added for 1 hour at 37° C. Adequate controls were used for each reagent.

Experimental Results

Propagation of Virus

Sections of control and inoculated spleen, lung, and amnio-allantoic membrane, stained with hematoxylin and eosin, showed considerable, apparently non-specific necrosis, and for this reason these tissues were not selected for serial passage. However, in four different individual experiments the various tissues mentioned were inoculated with the tissue culture virus and in every instance eosinophilic intranuclear inclusions were found. These bodies were identical with those previously described in fetal horse tissue.[4] Typical inclusions were present in the lining cells of the small bronchi (Fig. 1), but they were not numerous. Scattered and very infrequent inclusions were found in reticuloendothelial cells of the spleen and in certain cells of the connective tissue of the amnio-allantois.

In contrast to the other tissues studied, the epithelial lining of the fallopian tube was maintained *in vitro* in an excellent state of preservation (Fig. 2). Numerous inclusions (Fig. 3) were present regularly in representative sections of each of 8 serial passages, the last representing a dilution of virus of $10^{-41.4}$. No significant difference in necrosis could be detected in either the control or inoculated cultures.

Infectivity of Tissue Culture Virus for Fetal Horse Tissue

Fetal horse lung and spleen maintained in the same way as the adult tissues were inoculated with 6th passage fallopian tube material and incubated for 72 hours. Preparations stained with hematoxylin and eosin showed numerous intranuclear inclusions in both tissues and were identical with those previously described.[4] The inclusions were exceptionally abundant in the spleen and for this reason this tissue was used in the complement-fixation procedure, the final dilution of virus being 1×10^{-40}.

Complement-Fixing Activity of Tissue Culture Virus

Table I shows a comparison of the complement-fixing capacity of tissue culture material and control antigens. The fallopian tube antigen passage 8 was unsatisfactory because of its pronounced anti-complementary properties. Centrifugation and dilution failed to remove this

TABLE I

Complement Fixation of Tissue Culture Virus

Antigen	Passage no.	Dilution relative to original virus preparation	Dilution of antigens				
			¼	⅛	1/16	1/32	1/64
Original virus-containing tissue	0	½	4+	4+	4+	4+	+
Fetal horse spleen	16	$10^{-29.4}$	4+	+	−	−	−
Fallopian tube	6	$10^{-38.5}$	3+	−	−	−	−
Fallopian tube	Control of 6		−	−	−	−	−
Fetal horse spleen inoculated with passage 6	7	10^{-40}	A.C.	A.C.	A.C.	3+	+
Fetal horse spleen	Control of 7		A.C.	−	−	−	−
Fallopian tube	8	$10^{-41.4}$	A.C.	A.C.	A.C.	A.C.	A.C.

A.C. = anticomplementary.

anti-complementary factor. The dilution of tissue culture virus given is relative to the original virus inoculum noted previously.[4] It is clear from the data that several of the tissue culture antigens fixed complement at a significant titer. The titrations are expressed in terms of initial dilution of this test material before the addition of the various reagents. The titers with 1:10 dilution of the test serum are recorded. For reasons of brevity the various controls and standards are not indicated in Table I.

Discussion and Summary

According to the histologic and serologic data presented in this report, it is clear that the virus of equine abortion can be propagated serially in at least one type of adult horse tissue—the epithelium of the fallopian tube. Intranuclear inclusions have been demonstrated in this tissue and in lung, spleen, and amnio-allantoic membrane. The presence of inclusions in fallopian tube is apparently unique. Another example of viral infection of placental tissues has been documented previously.[7]

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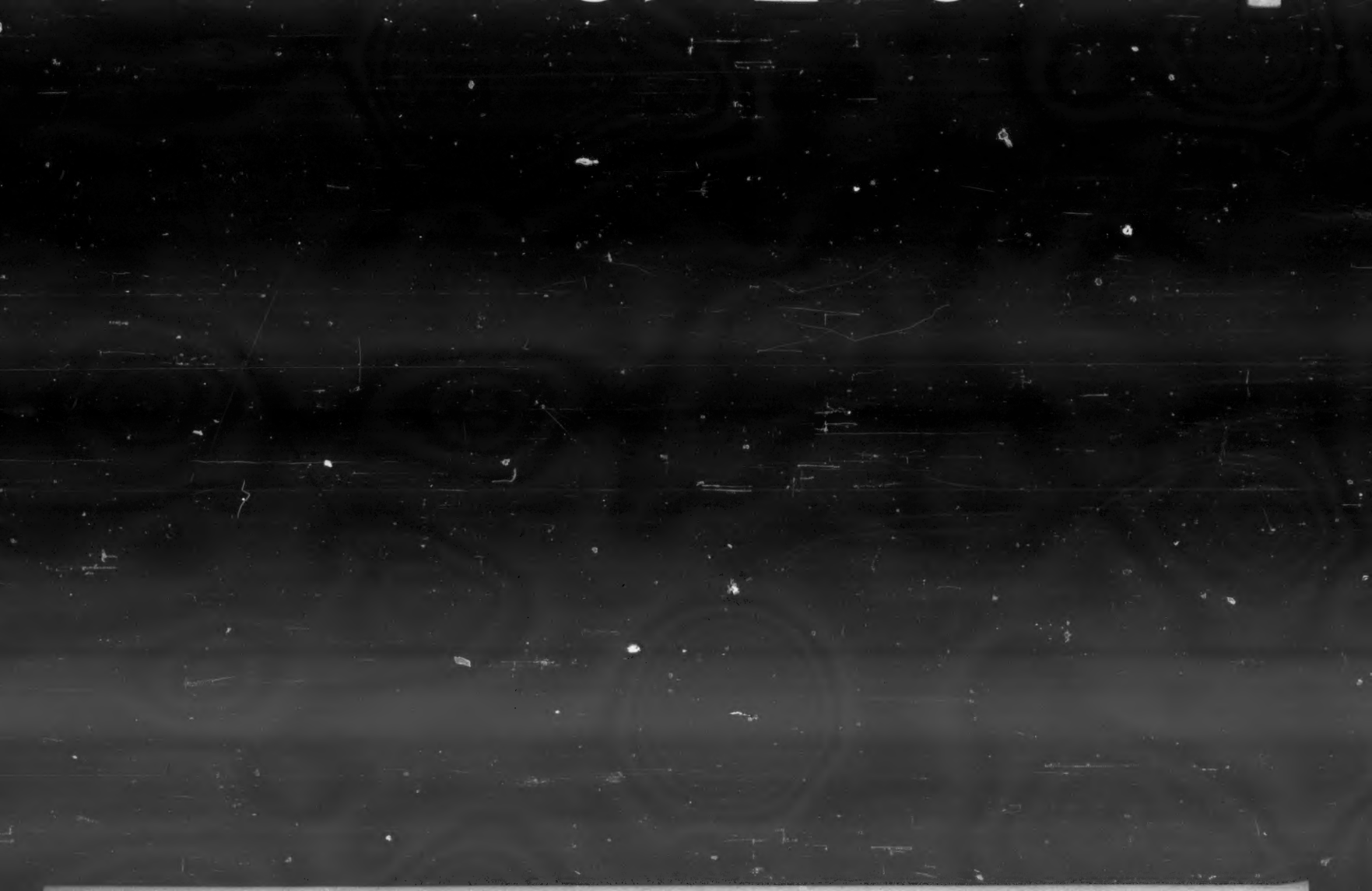
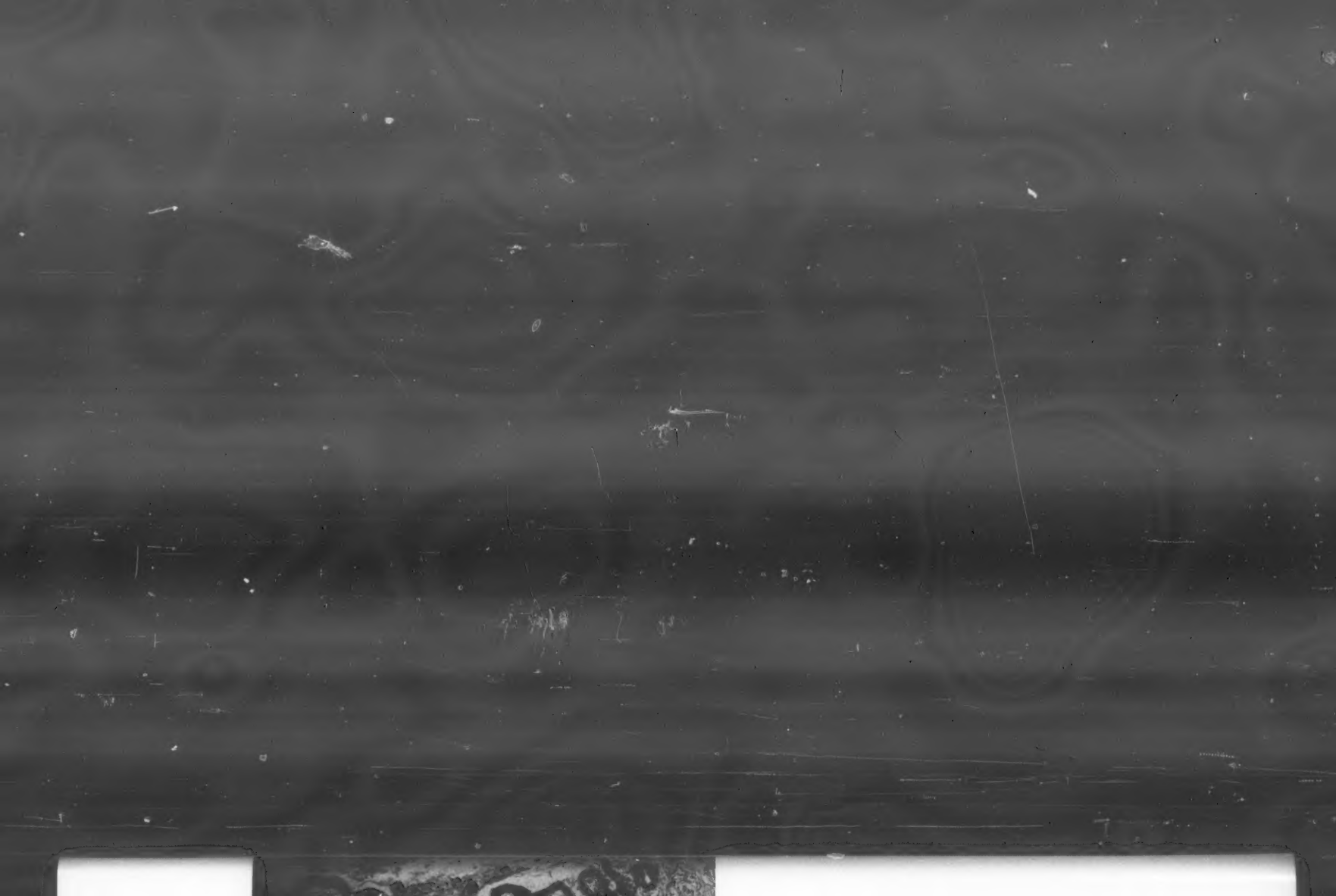
LEGENDS FOR FIGURES

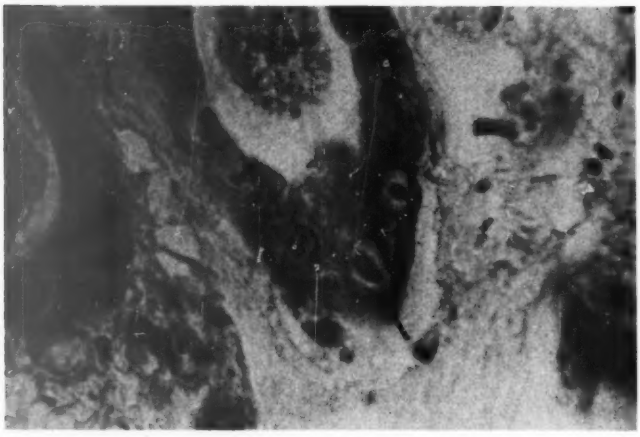
Fig. 1. Inoculated adult equine lung maintained for 48 hours by the flask method, fixed in Zenker-acetic acid fluid, and stained by hematoxylin and eosin. Several characteristic inclusions are clearly shown in the epithelium of a small bronchus. Oil immersion. × 727.

Fig. 2. Lining of inoculated adult equine fallopian tube maintained for 72 hours, passage 4, fixed in Zenker-acetic acid fluid, and stained with hematoxylin and eosin. The papillary projections are supported by a small amount of connective tissue. The lining cells are well preserved. × 50.

Fig. 3. Higher magnification of Figure 2. Numerous clear inclusions may be seen in the epithelial cells, some of which are indicated by arrows. This section is typical of the lesions found in the eight serial passages. Oil immersion. × 727.







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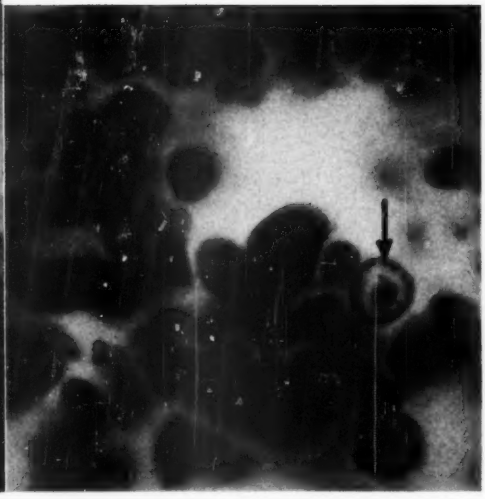

3

INDEX TO VOLUME XXXI

1955

INDEX OF SUBJECTS

3-Acetyl pyridine—Pathologic effects of antimetabolites. I. Acute lesions in the hypothalamus, peripheral ganglia, and adrenal medulla caused by . . . and prevented by nicotinamide. (*Hicks:* January-February) 189

Actinomycin D—Carcinolytic action of antibiotics: puromycin and . . . . (*Farber:* May-June) 582*

Adenoma—Influence of the intensity of exposure upon the number of pulmonary adenomas initiated by a single *in vitro* exposure to ultraviolet irradiation. (*Rogers:* May-June) 582*

Adrenal gland—Adrenal necrosis and thrombosis in routine necropsies. (*Plaut:* January-February) 93

——Hyperadrenocorticism: a histochemical study of surgically resected adrenal glands. (*Cohen, Chapman, and Castleman:* May-June) . . 570*

——Pathologic effects of antimetabolites. I. Acute lesions in the hypothalamus, peripheral ganglia, and adrenal medulla caused by 3-acetyl pyridine and prevented by nicotinamide. (*Hicks:* January-February) 189

Adrenal necrosis and thrombosis in routine necropsies. (*Plaut:* January-February) 93

Alloxan—Cytology of islet cells in . . . diabetic rabbits. (*Bencosme:* November-December) 1149

Alpha cells—Effect of large doses of growth hormone on the pancreatic . . . in the rat. (*Volk and Goldner:* May-June) 569*

Altitude—Susceptibility of endocardium of . . . rats to various bacteria. (*Highman and Allland:* May-June) 603*

Amaurotic family idiocy—Morphologic evolution of . . .: the protracted phase of the disease. (*Aronson, Volk, and Epstein:* July-August) . 609

American Association of Pathologists and Bacteriologists—Proceedings of the . . . . (May-June) 557

Amino acid—Histopathology of . . . deficiencies. IV. Tryptophan. (*Scott:* November-December) 1111

Aminonucleoside—Nephrotic syndrome induced in rats by . . . . (*Craig:* May-June) 591*

Amyloid—Electrophoretic analysis of . . . . (*Wagner:* May-June) . . 573*

Analytical pathology. II. Histopathologic demonstration of glomerular-localizing antibodies in experimental glomerulonephritis. (*Mellors, Arias-Stella, Siegel, and Pressman:* May-June, 592*; July-August) 687

Aneurysm—Bacterial aortitis and mycotic . . . of the aorta: a report of twelve cases. (*Parkhurst and Decker:* September-October) 821

Aneurysms of the aorta: a report of 358 cases. (*Brindley and Stembridge:* May-June) 576*

Antibodies—Analytical pathology. II. Histopathologic demonstration of glomerular-localizing . . . in experimental glomerulonephritis. (*Mellors, Arias-Stella, Siegel, and Pressman:* May-June, 592*; July-August) 687

Antigen—Detection of soluble carcinoma . . . by use of the Schultz-Dale test. (*Makari:* May-June) 581*

——Systemic effects of carbon particles coated with . . . . (*Garcia-Caceres and Black-Schaffer:* May-June) 601*

Antimetabolites—Pathologic effects of . . . . I. Acute lesions in the hypothalamus, peripheral ganglia, and adrenal medulla caused by 3-acetyl pyridine and prevented by nicotinamide. (*Hicks:* January-February) 189

Aorta—Aneurysms of the . . .: a report of 358 cases. (*Brindley and Stembridge:* May-June) 576*

——Bacterial aortitis and mycotic aneurysm of the . . . : a report of twelve cases. (*Parkhurst and Decker:* September-October) . . . 821

* Abstract of paper presented at the meeting of the American Association of Pathologists and Bacteriologists held at Houston, Texas, April 7, 8, and 9, 1955.

——Intimal repair of the . . . of the rabbit following experimental trauma. (*Prior and Hutter:* January-February) 107
Application of thin sections to the study of glomerular changes in glomerulonephritis. (*Grishman and Churg:* May-June) 602*
Arteries—Experimental arteriopathy: spontaneous, epinephrine-thyroxine, and cholesterol-induced forms. (*Oester, Davis, and Friedman:* July-August) 717
Arteriosclerosis—Coronary arteriosclerotic heart disease in the younger age group; its greater frequency among an increasingly older necropsy population. (*Saphir and Ohringer:* May-June) 575*
——Rôle of pulmonary hypertension and thrombo-embolism in the production of pulmonary . . . . (*O'Neal and Thomas:* May-June) . 578*
Arthropathy—Hemophilic . . . in dogs. (*Swanton and Brinkhous:* May-June) 596*
Asbestos—Granulomatous pneumonitis caused by short-fibered . . . dust (chrysotile). (*Gross, Westrick, and McNerney:* May-June) . . . 579*
Aschoff body—Clinical significance of the . . . based on morphologic and histochemical observations. (*Tedeschi, Wagner, and Pani:* May-June) 576*
Association of masculinizing ovarian hilus cell tumors, ovarian stromal hyperplasia, and lutein-like cell proliferation. (*Sternberg:* May-June) 571*
Atherosclerosis—Experimental . . . in the rat. (*Bragdon and Mickelsen:* September-October) 965
Atomic bomb—Pathology of swine exposed to total body gamma radiation from an . . . source. (*Tullis, Lamson, and Madden:* January-February) 41
Atypical cirrhosis in the duck produced by methylcholanthrene. (*Rigdon:* May-June) 461

Bacterial aortitis and mycotic aneurysm of the aorta: a report of twelve cases. (*Parkhurst and Decker:* September-October) 821
Bacterial endocarditis produced by the tubercle bacillus. (*Russell and Berthrong:* May-June) 577*
Basic pathologic forms of cerebral palsy. (*Towbin:* May-June) . . . 567*
Basophilic degeneration of the myocardium. (*Scotti:* May-June) . . 606*
Blastomycetes—Morphologic variation in tissue of the organisms of the blastomycoses and of histoplasmosis. (*Moore:* November-December) 1049
Blood vessels—Experimental atherosclerosis in the rat. (*Bragdon and Mickelsen:* September-October) 965
——Periarteritis nodosa limited to the pulmonary circulation. (*Braunstein:* September-October) 837
——Selective renal vasospasm and ischemic renal necrosis produced experimentally with staphylococcal toxin. Observations on the pathogenesis of bilateral cortical necrosis. (*Thal:* March-April) . . 233
Bone marrow—A strain of metastatic epithelial cells derived from long-term tissue culture of human . . . . (*Berman, Stulberg, and Ruddle:* May-June) 580*
Bovine—Pathogenesis of . . . ocular squamous cell carcinoma: histopathologic and clinical studies. (*Wynne and Russell:* May-June) . 584*
Brain—Basic pathologic forms of cerebral palsy. (*Towbin:* May-June) . 567*
——Embolization of cerebral tissue to lungs following severe head injury. (*McMillan:* May-June) 579*
——Experimental cerebral mucormycosis in diabetic rabbits. (*Bauer, Flanagan, and Sheldon:* May-June) 600*
——Histoplasmosis of the central nervous system. (*Shapiro, Lux, and Sprofkin:* March-April) 319
——Lesions of the corpus callosum following blunt mechanical trauma to the head. (*Lindenberg, Fisher, Durlacher, Lovitt, and Freytag:* March-April) 297

——Morphologic evolution of amaurotic family idiocy: the protracted phase of the disease. (*Aronson, Volk, and Epstein:* July-August) . 609
——Sarcoma arising in glioblastoma of the . . . . (*Feigin and Gross:* July-August) 633
Breast—Increased spontaneous occurrence of fibroadenomas of the . . . in the albino rat influenced by a high fat diet. (*Benson, Lev, and Grand:* May-June) 583*
——Involvement of internal mammary lymph nodes in carcinoma of the . . .: studies of the extended radical mastectomy. (*Wyatt, Sugarbaker, and Stanton:* January-February) 143
——Tissue culture of human . . . carcinoma. (*Orr and McSwain:* January-February) 125
Brucella—Immunization against . . . infection. V. Histopathologic appraisal of immunity induced in mice by a streptomycin-dependent mutant of . . . *melitensis*. (*Elberg, Steiner, and Doll:* November-December) 1065
Burns—On the pathogenesis of death due to burning of the skin. (*Rosenthal, Finamore, Hunter, Hunter, and Roman:* May-June) 568*

Cancer of the cervix uteri: a review of 169 necropsied cases. (*Marcial-Rojas and Meigs:* November-December) 1077
Carcinolytic action of antibiotics: puromycin and actinomycin D. (*Farber:* May-June) 582*
Carcinoma—A strain of metastatic epithelial cells derived from long-term tissue culture of human bone marrow. (*Berman, Stulberg, and Ruddle:* May-June) 580*
——Cancer of the cervix uteri: a review of 169 necropsied cases. (*Marcial-Rojas and Meigs:* November-December) 1077
——Cell types and histologic patterns in . . . of the lung: observations on the significance of tumors containing more than one type of cell. (*Olcott:* November-December) 975
——Detection of soluble . . . antigen by use of the Schultz-Dale test. (*Makari:* May-June) 581*
——Endometrial hyperplasia and . . . produced by estrogen. (*Meissner and Sommers:* May-June) 571*
——Involvement of internal mammary lymph nodes in . . . of the breast: studies of the extended radical mastectomy. (*Wyatt, Sugarbaker, and Stanton:* January-February) 143
——Pathogenesis of bovine ocular squamous cell . . .: histopathologic and clinical studies. (*Wynne and Russell:* May-June) 584*
——Signet-ring cell . . . of the urinary bladder. (*Saphir:* March-April) . 223
——Three-dimensional analysis of hepatocellular . . . . (*Elias and Popper:* May-June) 596*
——Tissue culture of human breast . . . . (*Orr and McSwain:* January-February) 125
Cardiopneumonopexy—Retrocardiac, intercoronary, and transpleural components in experimentally produced collateral circulation to the heart. (*Kline, Bloomer, Stern, and Liebow:* May-June) 574*
Cell types and histologic patterns in carcinoma of the lung: observations on the significance of tumors containing more than one type of cell. (*Olcott:* November-December) 975
Central nervous system—Histoplasmosis of the . . . . (*Shapiro, Lux, and Sprofkin:* March-April) 319
Cerebral palsy—Basic pathologic forms of . . . . (*Towbin:* May-June) . 567*
Chemotherapy—Resistance to quadruple combination . . . in leukemia L1210 in mice. (*Nadel and Hilgar:* May-June) 605*
Chicken—Newcastle disease virus in cultures of chick embryo tissues: its multiplication, titration, and cytopathogenicity. (*Mason and Kaufman:* September-October) 883
——Pathology of poliomyelitis in the chick embryo. (*Love and Roca-Garcia:* September-October) 901

Chlorophyllin injection in pregnant mice. (*Glaubach and Antopol:* May-June) 601*
Choline—Fat emboli in glomerular capillaries of . . .-deficient rats and of patients with diabetic glomerulosclerosis. (*Hartroft:* May-June) . 381
Chromosomes—Further studies in quantitative cytopathology: optical measurements of the organic mass and the nucleoprotein mass of . . . in normal and neoplastic cells. (*Mellors and Ortega:* May-June) . 604*
Chylothorax—Intrathoracic angiomyomatous hyperplasia associated with chronic . . . . (*Laipply and Sherrick:* May-June) 599*
Cirrhosis—Atypical . . . in the duck produced by methylcholanthrene. (*Rigdon:* May-June) 461
——Coarse nodular . . . produced by ethionine. (*Popper, Clearkin, Kent, Parthasarathy, and Bruce:* May-June) 594*
——Congenital . . . of the liver with kernicterus: report of two cases in siblings with a discussion of the relationship to so-called neonatal hepatitis and to iso-immunization disease. (*Ehrlich and Ratner:* November-December) 1013
——Histogenesis of hepatic . . . studied by the three-dimensional approach. (*Popper and Elias:* May-June) 405
Clinical significance of the Aschoff body based on morphologic and histochemical observations. (*Tedeschi, Wagner, and Pani:* May-June) . 576*
Coarse nodular cirrhosis produced by ethionine. (*Popper, Clearkin, Kent, Parthasarathy, and Bruce:* May-June) 594*
Collagen diseases—Morphologic evidence of the hypersensitive pathogenesis of the lesions of experimental hypersensitivity and the . . . . (*More and Movat:* May-June) 562*
Comparison of histochemical properties of normal and neoplastic squamous epithelium. (*Foraker:* May-June) 581*
Congenital cirrhosis of the liver with kernicterus: report of two cases in siblings with a discussion of the relationship to so-called neonatal hepatitis and to iso-immunization disease. (*Ehrlich and Ratner:* November-December) 1013
Copper—Histochemical demonstration of . . . in a case of hepatolenticular degeneration. (*Green:* May-June) 545
Coronary arteriosclerotic heart disease in the younger age group; its greater frequency among an increasingly older necropsy population. (*Saphir and Ohringer:* May-June) 575*
Coronary artery disease—Pathophysiologic evaluation in dogs of two surgical procedures used in patients with occlusive . . . . (*Maniglia and Bakst:* May-June) 574*
Corpus callosum—Lesions of the . . . following blunt mechanical trauma to the head. (*Lindenberg, Fisher, Durlacher, Lovitt, and Freytag:* March-April) 297
Correlation of glomerular filtration rate with histopathologic changes in glomeruli as seen in needle biopsy of the kidney. (*Howe and Parrish:* May-June) 603*
Cortical necrosis—Selective renal vasospasm and ischemic renal necrosis produced experimentally with staphylococcal toxin. Observations on the pathogenesis of bilateral . . . . (*Thal:* March-April) 233
Cortisone—Glomerular ultraviolet absorptions after . . . therapy, simulating diabetes mellitus. (*Sommers:* May-June) 570*
——Influence of . . . on the development of the experimental silica granuloma in the rat. (*Clark:* May-June) 563*
——Nodular and fatty glomerular lesions in rabbits on . . . . (*Wilens and Stumpf:* March-April) 275
——Pancreatic lesions and peripancreatic fat necrosis in . . .-treated rabbits. (*Stumpf and Wilens:* May-June) 563*
Cortisone in experimental histoplasmosis. (*Vogel, Michael, and Timpe:* May-June) 535
Cretinism—Structural features of goiters in sporadic cretins. (*Milles:* November-December) 997

Cystic disease of the lungs: a consequence of the healing of acute disseminated pulmonary tuberculosis. (*Berthrong and Ganem:* May-June) 601*
Cystinosis (Lignac-Fanconi disease). (*Gatzimos, Schulz, and Newnum:* May-June, 567*; September-October) 791
Cytology of islet cells in alloxan diabetic rabbits. (*Bencosme:* November-December) 1149

Destructive effects of DL-ethionine on the pancreas, stomach, and submaxillary glands. (*Loring and Hartley:* May-June) 521
Detection of soluble carcinoma antigen by use of the Schultz-Dale test. (*Makari:* May-June) 581*
Diabetes—Cytology of islet cells in alloxan diabetic rabbits. (*Bencosme:* November-December) 1149
——Experimental cerebral mucormycosis in diabetic rabbits. (*Bauer, Flanagan, and Sheldon:* May-June) 600*
Diabetes mellitus—Fat emboli in glomerular capillaries of choline-deficient rats and of patients with diabetic glomerulosclerosis. (*Hartroft:* May-June) 381
——Fat embolism in diabetic patients without physical trauma. (*Kent:* May-June) 399
——Histochemical observations on hyalinized islets of Langerhans in . . . . (*Toreson and Rinehart:* May-June) 569*
——Histopathology of experimental glomerular lesions simulating human diabetic glomerulosclerosis. (*Bloodworth and Hamwi:* January-February) 167
Diet—Increased spontaneous occurrence of fibroadenomas of the breast in the albino rat influenced by a high fat . . . . (*Benson, Lev, and Grand:* May-June) 583*
Dietary deficiencies—Histopathology of amino acid deficiencies. IV. Tryptophan. (*Scott:* November-December) 1111
DL-ethionine—The destructive effects of . . . on the pancreas, stomach, and submaxillary glands. (*Loring and Hartley:* May-June) . . . 521
Dog—Effects of prolonged experimental hypertension in the . . . with a study of periodic renal biopsies over a seven-year period. (*Dammin, Goldman, Schroeder, and Pace:* May-June) 587*
——Hemangiopericytoma in the . . . . (*Mulligan:* July-August) . . . 773
——Hemophilic arthropathy in dogs. (*Swanton and Brinkhous:* May-June) 596*
——Pathophysiologic evaluation in dogs of two surgical procedures used in patients with occlusive coronary artery disease. (*Maniglia and Bakst:* May-June) 574*
Duck—Atypical cirrhosis in the . . . produced by methylcholanthrene. (*Rigdon:* May-June) 461
——Neurogenic tumors in the . . .: an experimental study. (*Rigdon:* May-June) 583*
——The pathology of infectious serositis of ducks. (*Dougherty, Saunders, and Parsons:* May-June) 475

Effect of audiogenic stimuli on wound healing in DBA/1 mice. (*Spain, Molomut, and Riess:* May-June) 607*
Effect of feeding irradiated trichinella larvae on production of immunity to re-infection. (*Gould, Gomberg, Bethell, Villella, and Hertz:* May-June) 561*
Effect of hyaluronidase antagonists on the reaction of the host to viral infection. (*Pearce:* May-June) 562*
Effect of large doses of growth hormone on the pancreatic alpha cells in the rat. (*Volk and Goldner:* May-June) 569*
Effects of massive whole body radiation in rats. (*Koletsky:* May-June) . 568*
Effects of prolonged experimental hypertension in the dog with a study of periodic renal biopsies over a seven-year period. (*Dammin, Goldman, Schroeder, and Pace:* May-June) 587*

Electrophoretic analysis of amyloid. (*Wagner:* May-June) 573*
Embolization of cerebral tissue to lungs following severe head injury. (*McMillan:* May-June) 579*
Encephalitis—Mumps . . .: pathology and pathogenesis. (*Donohue, Playfair, and Whitaker:* May-June) 565*
Endocarditis—Bacterial . . . produced by the tubercle bacillus. (*Russell and Berthrong:* May-June) 577*
——Susceptibility of endocardium of altitude rats to various bacteria. (*Highman and Altland:* May-June) 603*
Endometrial hyperplasia and carcinoma produced by estrogen. (*Meissner and Sommers:* May-June) 571*
Enzymes—Histochemical localization of certain oxidative . . . during the production of infarcts of the rat kidney. (*Peters and Farber:* May-June) 590*
——Histochemical localization of enzymatic activities in various portions of the mammalian nephron. (*Wachstein and Meisel:* May-June) . 588*
——Improved method for the histochemical localization of oxidative . . . in the kidney with tetrazolium. (*Farber:* May-June) 589*
Equine abortion—Susceptibility *in vitro* of adult horse tissue to . . . virus: previously undescribed lesions. (*Randall:* November-December) . 1165
Estrogen—Endometrial hyperplasia and carcinoma produced by . . . . (*Meissner and Sommers:* May-June) 571*
Ethionine—Coarse nodular cirrhosis produced by . . . . (*Popper, Clearkin, Kent, Parthasarathy, and Bruce:* May-June) 594*
Experimental arteriopathy: spontaneous, epinephrine-thyroxine, and cholesterol-induced forms. (*Oester, Davis, and Friedman:* July-August) 717
Experimental aspiration pneumonia: inflammatory and reparative changes produced by intratracheal injections of autologous gastric juice and hydrochloric acid. (*Morgan:* May-June) 579*
Experimental atherosclerosis in the rat. (*Bragdon and Mickelsen:* September-October) 965
Experimental carcinogenesis of the skin in mice: dose-response relationships. (*McTurk, Eckardt, Smith, and Cooper:* May-June) 582*
Experimental cerebral mucormycosis in diabetic rabbits. (*Bauer, Flanagan, and Sheldon:* May-June) 600*
Experimental moniliasis in mice. (*Adriano and Schwarz:* September-October) 859
Eye—Pathogenesis of bovine ocular squamous cell carcinoma: histopathologic and clinical studies. (*Wynne and Russell:* May-June) . 584*

Factors influencing metachromasy in solutions. (*Schoenberg and Orbison:* May-June) 573*
Fat emboli in glomerular capillaries of choline-deficient rats and of patients with diabetic glomerulosclerosis. (*Hartroft:* May-June) . . 381
Fat embolism—Fat emboli in glomerular capillaries of choline-deficient rats and of patients with diabetic glomerulosclerosis. (*Hartroft:* May-June) 381
——in diabetic patients without physical trauma. (*Kent:* May-June) . 399
Fibrinoid—Morphologic changes in rabbits following the intravenous administration of meningococcal toxin. II. Two appropriately spaced injections; the rôle of . . . in the generalized Shwartzman reaction. (*Brunson, Thomas, and Gamble:* July-August) 655
Fibroadenoma—Increased spontaneous occurrence of fibroadenomas of the breast in the albino rat influenced by a high fat diet. (*Benson, Lev, and Grand:* May-June) 583*
Fibrocystic disease—The pathogenesis of . . . of the pancreas. Study of the ducts by serial sections. (*Allen and Baggenstoss:* March-April) . 337
Fine structure of the renal glomerulus. Histochemical and electron microscopic observations. (*Rinehart, Farquhar, and Gould:* May-June) 587*

Focal myocytolysis of the heart. (*Schlesinger and Reiner:* May-June) . 443
Further studies in quantitative cytopathology: optical measurements of the organic mass and the nucleoprotein mass of chromosomes in normal and neoplastic cells. (*Mellors and Ortega:* May-June) . . 604*

Gamma rays—Pathology of swine exposed to total body gamma radiation from an atomic bomb source. (*Tullis, Lamson, and Madden:* January-February) 41
Giant cells—Neonatal jaundice with giant cell transformation of the hepatic parenchyma. (*Smetana and Johnson:* July-August) . . . 747
Glioblastoma—Sarcoma arising in . . . of the brain. (*Feigin and Gross:* July-August) 633
Glioma—The nature of gliomas as revealed by animal experimentation. (*Zimmerman:* January-February) 1
Glomerular lesions and the nephrotic syndrome in rabbits given saccharated iron oxide intravenously. (*Ellis:* May-June) 591*
Glomerular ultraviolet absorptions after cortisone therapy, simulating diabetes mellitus. (*Sommers:* May-June) 570*
Glomerulonephritis: a correlation of histopathology in needle biopsies of the kidney with the clinical course. (*Howe and Parrish:* May-June) 594*
——Analytical pathology. II. Histopathologic demonstration of glomerular-localizing antibodies in experimental . . . . (*Mellors, Arias-Stella, Siegel, and Pressman:* May-June, 592*; July-August) . . 687
——Application of thin sections to the study of glomerular changes in . . . . (*Grishman and Churg:* May-June) 602*
Glomerulosclerosis—Fat emboli in glomerular capillaries of choline-deficient rats and of patients with diabetic . . . . (*Hartroft:* May-June) 381
——Histopathology of experimental glomerular lesions simulating human diabetic . . . . (*Bloodworth and Hamwi:* January-February) 167
Glomerulus—Correlation of glomerular filtration rate with histopathologic changes in glomeruli as seen in needle biopsy of the kidney. (*Howe and Parrish:* May-June) 603*
——Fine structure of the renal . . . . Histochemical and electron microscopic observations. (*Rinehart, Farquhar, and Gould:* May-June) . 587*
——Ischemic glomeruli and their significance for glomerular structure. (*McManus:* May-June) 589*
Goiter—Structural features of goiters in sporadic cretins. (*Milles:* November-December) 997
Granulomatous pneumonitis caused by short-fibered asbestos dust (chrysotile). (*Gross, Westrick, and McNerney:* May-June) . . . 579*
Growth hormone—Effect of large doses of . . . on the pancreatic alpha cells in the rat. (*Volk and Goldner:* May-June) 569*
Guinea-pig—Serum elpherograms in experimental nephropathies of guinea-pigs. (*Pollak and Cruz:* May-June) 573*

Healed primary complex in histoplasmosis. (*Straub and Schwarz:* May-June) 599*
Heart—Bacterial endocarditis produced by the tubercle bacillus. (*Russell and Berthrong:* May-June) 577*
——Basophilic degeneration of the myocardium. (*Scotti:* May-June) . 606*
——Coronary arteriosclerotic . . . disease in the younger age group; its greater frequency among an increasingly older necropsy population. (*Saphir and Ohringer:* May-June) 575*
——Focal myocytolysis of the . . . . (*Schlesinger and Reiner:* May-June) 443
——Pathologic and physiologic effects of hemorrhagic shock on the . . . . (*Hackel:* May-June) 576*

——Pathophysiologic evaluation in dogs of two surgical procedures used in patients with occlusive coronary artery disease. (*Maniglia and Bakst:* May-June) 574*
——Retrocardiac, intercoronary, and transpleural components in experimentally produced collateral circulation to the . . . . (*Kline, Bloomer, Stern, and Liebow:* May-June) 574*
——Rheumatic activity in auricular appendages removed at mitral valvoplasty. (*Clark and Anderson:* September-October) 809
——Succinic dehydrogenase activity in myocardial infarction and in induced myocardial necrosis. (*Wachstein and Meisel:* March-April) . 353
——Susceptibility of endocardium of altitude rats to various bacteria. (*Highman and Altland:* May-June) 603*
Hemangiopericytoma in the dog. (*Mulligan:* July-August) 773
Hemophilic arthropathy in dogs. (*Swanton and Brinkhous:* May-June) . 596*
Hemorrhagic fever—Pathology of . . . . A comparison of the findings — 1951 and 1952. (*Steer:* March-April) 201
Hepatitis—Congenital cirrhosis of the liver with kernicterus: report of two cases in siblings with a discussion of the relationship to so-called neonatal . . . and to iso-immunization disease. (*Ehrlich and Ratner:* November-December) 1013
Hepatolenticular degeneration—Histochemical demonstration of copper in a case of . . . . (*Green:* May-June) 545
Hepatorenal syndrome—Pathogenetic studies in the experimental . . . using micro-angiography in the agonal phase. (*Crowson, More, and King:* May-June) 593*
Heterotopic ossification in intestinal neoplasms. (*Van Patter and Whittick:* January-February) 73
High molecular weight acidic polymers—Morphologic changes in rabbits following the intravenous administration of meningococcal toxin. III. The effects produced by endotoxin in association with certain . . . . (*Brunson, Davis, and Thomas:* July-August) 669
Hilus cell tumors—Association of masculinizing ovarian . . ., ovarian stromal hyperplasia, and lutein-like cell proliferation. (*Sternberg:* May-June) 571*
Histochemical demonstration of copper in a case of hepatolenticular degeneration. (*Green:* May-June) 545
Histochemical localization of certain oxidative enzymes during the production of infarcts of the rat kidney. (*Peters and Farber:* May-June) 590*
Histochemical localization of enzymatic activities in various portions of the mammalian nephron. (*Wachstein and Meisel:* May-June) . . 588*
Histochemical observations on hyalinized islets of Langerhans in diabetes mellitus. (*Toreson and Rinehart:* May-June) 569*
Histochemical studies on the activation of tissue alkaline phosphatase and 5-nucleotidase. (*Freiman:* May-June) 572*
Hist ;enesis of hepatic cirrhosis studied by the three-dimensional approach. (*Popper and Elias:* May-June) 405
Histopathologic study of the small intestine of immature non-immunized mice infected with Trichinella spiralis. (*Larsh, Jeffries, and Race:* May-June) 561*
Histopathology of amino acid deficiencies. IV. Tryptophan. (*Scott:* November-December) 1111
Histopathology of experimental glomerular lesions simulating human diabetic glomerulosclerosis. (*Bloodworth and Hamwi:* January-February) 167
Histoplasma capsulatum—Morphologic variation in tissue of the organisms of the blastomycoses and of histoplasmosis. (*Moore:* November-December) 1049
Histoplasmosis—Cortisone in experimental . . . . (*Vogel, Michael, and Timpe:* May-June) 535
——Healed primary complex in . . . . (*Straub and Schwarz:* May-June) 599*

——Relation of splenic calcification to . . . . (*Schwarz, Silverman, Straub, Levine, and Adriano:* May-June) 606*
Histoplasmosis of the central nervous system. (*Shapiro, Lux, and Sprofkin:* March-April) 319
Hodgkin's disease—Mortality in relation to histology in . . . . (*Smetana and Cohen:* May-June) 597*
Horse—Susceptibility *in vitro* of adult . . . tissue to equine abortion virus: previously undescribed lesions. (*Randall:* November-December) . 1165
Human lung in leukemia: observations on alveolar capillary leukostasis with reference to pathologic physiology. (*Old, Smith, and Grampa:* May-June) 605*
Hyaluronidase—Effect of . . . antagonists on the reaction of the host to viral infection. (*Pearce:* May-June) 562*
Hyperadrenocorticism: a histochemical study of surgically resected adrenal glands. (*Cohen, Chapman, and Castleman:* May-June) . . 570*
Hyperplasia—Intrathoracic angiomyomatous . . . associated with chronic chylothorax. (*Laipply and Sherrick:* May-June) 599*
Hypersensitivity—Morphologic evidence of the hypersensitive pathogenesis of the lesions of experimental . . . and the collagen diseases. (*More and Movat:* May-June) 562*
Hypertension—Effects of prolonged experimental . . . in the dog with a study of periodic renal biopsies over a seven-year period. (*Dammin, Goldman, Schroeder, and Pace:* May-June) 587*
——Reduction of post-nephrectomy . . . by homogenous renal transplant mediated by means other than desalting and dehydration. (*Muirhead, Stirman, and Lesch:* May-June) 585*
——Rôle of pulmonary . . . and thrombo-embolism in the production of pulmonary arteriosclerosis. (*O'Neal and Thomas:* May-June) . . 578*
Hypervitaminosis D—Studies on . . . . (*Follis:* May-June) 568*
Hypophysis—Possible endocrine significance of hypophyseal tumors in man. (*Russfield and Reiner:* May-June) 572*
——Study of cell groups in the . . . and their relation to granular cell myoblastomas. (*Davis and Butt:* May-June) 566*
——Transplantation of the parakeet pituitary tumor. (*Schlumberger:* May-June) 583*
Hypothalamus—Pathologic effects of antimetabolites. I. Acute lesions in the . . ., peripheral ganglia, and adrenal medulla caused by 3-acetyl pyridine and prevented by nicotinamide. (*Hicks:* January-February) 189

Immunization against Brucella infection. V. Histopathologic appraisal of immunity induced in mice by a streptomycin-dependent mutant of *Brucella melitensis*. (*Elberg, Steiner, and Doll:* November-December) 1065
Improved method for the histochemical localization of oxidative enzymes in the kidney with tetrazolium. (*Farber:* May-June) 589*
Increased spontaneous occurrence of fibroadenomas of the breast in the albino rat influenced by a high fat diet. (*Benson, Lev, and Grand:* May-June) 583*
Infants—Pathologic findings associated with unexpected death in . . . and children. (*Arey:* May-June) 578*
Infarction—Succinic dehydrogenase activity in myocardial . . . and in induced myocardial necrosis. (*Wachstein and Meisel:* March-April) 353
Influence of cortisone on the development of the experimental silica granuloma in the rat. (*Clark:* May-June) 563*
Influence of the intensity of exposure upon the number of pulmonary adenomas initiated by a single *in vitro* exposure to ultraviolet irradiation. (*Rogers:* May-June) 582*
Injection corrosion studies of the vascular systems of normal and cirrhotic livers. (*Hales, Allan, and Hall:* May-June) 595*

Intestine—Heterotopic ossification in intestinal neoplasms. (*Van Patter and Whitlick:* January-February) 73
——Histopathologic study of the small . . . of immature non-immunized mice infected with *Trichinella spiralis*. (*Larsh, Jeffries, and Race:* May-June) 561*
Intimal repair of the aorta of the rabbit following experimental trauma. (*Prior and Hutter:* January-February) 107
Intrathoracic angiomyomatous hyperplasia associated with chronic chylothorax. (*Laipply and Sherrick:* May-June) 599*
Involvement of internal mammary lymph nodes in carcinoma of the breast: studies of the extended radical mastectomy. (*Wyatt, Sugarbaker, and Stanton:* January-February) 143
Irradiation—Effect of feeding irradiated trichinella larvae on production of immunity to re-infection. (*Gould, Gomberg, Bethell, Villella, and Hertz:* May-June) 561*
——Effects of massive whole body radiation in rats. (*Koletsky:* May-June) 568*
——Influence of the intensity of exposure upon the number of pulmonary adenomas initiated by a single *in vitro* exposure to ultraviolet . . . . (*Rogers:* May-June) 582*
——Mitochondrial changes in hepatic cells of x-irradiated mice. (*MacCardle and Congdon:* July-August) 725
——Pathology of swine exposed to total body gamma radiation from an atomic bomb source. (*Tullis, Lamson, and Madden:* January-February) 41
Ischemic glomeruli and their significance for glomerular structure. (*McManus:* May-June) 589*
Islet cells—Cytology of . . . in alloxan diabetic rabbits. (*Bencosme:* November-December) 1149

Karyomegaly—Systemic . . . . Case report of a heretofore undescribed disease entity. (*Moritz and Leuchtenberger:* May-June) 564*
Kernicterus—Congenital cirrhosis of the liver with . . .: report of two cases in siblings with a discussion of the relationship to so-called neonatal hepatitis and to iso-immunization disease. (*Ehrlich and Rainer:* November-December) 1013
Kidney—Analytical pathology. II. Histopathologic demonstration of glomerular-localizing antibodies in experimental glomerulonephritis. (*Mellors, Arias-Stella, Siegel, and Pressman:* May-June, 592*; July-August) 687
——Application of thin sections to the study of glomerular changes in glomerulonephritis. (*Grishman and Churg:* May-June) 602*
——Correlation of glomerular filtration rate with histopathologic changes in glomeruli as seen in needle biopsy of the . . . . (*Howe and Parrish:* May-June) 603*
——Effects of prolonged experimental hypertension in the dog with a study of periodic renal biopsies over a seven-year period. (*Dammin, Goldman, Schroeder, and Pace:* May-June) 587*
——Fat emboli in glomerular capillaries of choline-deficient rats and of patients with diabetic glomerulosclerosis. (*Hartroft:* May-June) . 381
——Fine structure of the renal glomerulus. Histochemical and electron microscopic observations. (*Rinehart, Farquhar, and Gould:* May-June) 587*
——Glomerular lesions and the nephrotic syndrome in rabbits given saccharated iron oxide intravenously. (*Ellis:* May-June) 591*
——Glomerulonephritis: a correlation of histopathology in needle biopsies of the . . . with the clinical course. (*Howe and Parrish:* May-June) 594*
——Histochemical localization of certain oxidative enzymes during the production of infarcts of the rat . . . . (*Peters and Farber:* May-June) 590*

——Histochemical localization of enzymatic activities in various portions of the mammalian nephron. (*Wachstein and Meisel:* May-June) 588*
——Histopathology of experimental glomerular lesions simulating human diabetic glomerulosclerosis. (*Bloodworth and Hamwi:* January-February) 167
——Improved method for the histochemical localization of oxidative enzymes in the . . . with tetrazolium. (*Farber:* May-June) . . . 589*
——Ischemic glomeruli and their significance for glomerular structure. (*McManus:* May-June) 589*
——Local renal Shwartzman phenomenon. (*Black-Schaffer and Garcia-Caceres:* May-June) 592*
——Nephrotic syndrome induced in rats by aminonucleoside. (*Craig:* May-June) 591*
——New approaches to the study of renal disorders. (*Oliver:* May-June) 592*
——Nodular and fatty glomerular lesions in rabbits on cortisone. (*Wilens and Stumpf:* March-April) 275
——Pathogenetic studies in the experimental hepatorenal syndrome using micro-angiography in the agonal phase. (*Crowson, More, and King:* May-June) 593*
——Prolonged functional survival of a renal homotransplant in man: morphologic observations with clinical correlation. (*Dammin, Hume, Merrill, Miller, and Thorn:* May-June) 586*
——Reduction of post-nephrectomy hypertension by homogenous renal transplant mediated by means other than desalting and dehydration. (*Muirhead, Stirman, and Lesch:* May-June) 585*
——Renal diseases as studied by serial . . . biopsy. (*Pirani, Muehrcke, and Kark:* May-June) 594*
——Renal Shwartzman phenomenon in trichinosis. (*Yesner:* May-June) 592*
——Selective renal vasospasm and ischemic renal necrosis produced experimentally with staphylococcal toxin. Observations on the pathogenesis of bilateral cortical necrosis. (*Thal:* March-April) . . 233
——Serum elpherograms in experimental nephropathies of guinea-pigs. (*Pollak and Crux:* May-June) 573*
——Symposium on new approaches to the study of renal disorders. (*Oliver:* May-June) 585*
——The rôle of oxyhemoglobin and its derivatives in the pathogenesis of experimental hemoglobinuric nephrosis. (*Lalich:* January-February) 153
Kwashiorkor in a white American male. (*Diamond:* May-June) . . 596*

Leprosy: pathologic changes observed in fifty consecutive necropsies. (*Powell and Swan:* November-December) 1131
Leptospirosis—Lesions of skeletal muscle in . . . . Review of reports and an experimental study. (*Laurain:* May-June) 501
Lesions of skeletal muscle in leptospirosis. Review of reports and an experimental study. (*Laurain:* May-June) 501
Lesions of the corpus callosum following blunt mechanical trauma to the head. (*Lindenberg, Fisher, Durlacher, Lovitt, and Freytag:* March-April) 297
Lesions of the intrahepatic portal radicles in Manson's schistosomiasis. (*Lichtenberg:* May-June, 604*; July-August) 757
Leukemia—A case of leukemic reticulo-endotheliosis in the rat. The relationship between this entity and Naegeli's monocytic myelogenous . . . . (*Harris, Shay, and Gruenstein:* March-April) . . . 367
——Human lung in . . .: observations on alveolar capillary leukostasis with reference to pathologic physiology. (*Old, Smith, and Grampa:* May-June) 605*
——Pattern of organ infiltrate in acute . . . of children. (*Cochran and Haut:* May-June) 597*

——Resistance to quadruple combination chemotherapy in . . . L1210 in mice. (*Nadel and Hilgar:* May-June) 605*
Leukemic reticulo-endotheliosis in the rat. The relationship between this entity and Naegeli's monocytic myelogenous leukemia. (*Harris, Shay, and Gruenstein:* March-April) 367
Liver—Atypical cirrhosis in the duck produced by methylcholanthrene. (*Rigdon:* May-June) 461
——Coarse nodular cirrhosis produced by ethionine. (*Popper, Clearkin, Kent, Parthasarathy, and Bruce:* May-June) 594*
——Congenital cirrhosis of the . . . with kernicterus: report of two cases in siblings with a discussion of the relationship to so-called neonatal hepatitis and to iso-immunization disease. (*Ehrlich and Ratner:* November-December) 1013
——Histogenesis of hepatic cirrhosis studied by the three-dimensional approach. (*Popper and Elias:* May-June) 405
——Injection corrosion studies of the vascular systems of normal and cirrhotic livers. (*Hales, Allan, and Hall:* May-June) 595*
——Lesions of the intrahepatic portal radicles in Manson's schistosomiasis. (*Lichtenberg:* May-June, 604*; July-August) 757
——Mitochondrial changes in hepatic cells of x-irradiated mice. (*MacCardle and Congdon:* July-August) 725
——Neonatal jaundice with giant cell transformation of the hepatic parenchyma. (*Smetana and Johnson:* July-August) 747
——Pathogenetic studies in the experimental hepatorenal syndrome using micro-angiography in the agonal phase. (*Crowson, More, and King:* May-June) 593*
——Three-dimensional analysis of hepatocellular carcinoma. (*Elias and Popper:* May-June) 596*
Local renal Shwartzman phenomenon. (*Black-Schaffer and Garcia-Caceres:* May-June) 592*
LS tumor, a reticulum cell sarcoma of C57BL/6 mice. (*Hathaway and Dowling:* May-June) 583*
Lung—Cell types and histologic patterns in carcinoma of the . . .: observations on the significance of tumors containing more than one type of cell. (*Olcott:* November-December) 975
——Cystic disease of the lungs: a consequence of the healing of acute disseminated pulmonary tuberculosis. (*Berthrong and Ganem:* May-June) 601*
——Embolization of cerebral tissue to lungs following severe head injury. (*McMillan:* May-June) 579*
——Experimental aspiration pneumonia: inflammatory and reparative changes produced by intratracheal injections of autologous gastric juice and hydrochloric acid. (*Moran:* May-June) 579*
——Granulomatous pneumonitis caused by short-fibered asbestos dust (chrysotile). (*Gross, Westrick, and McNerney:* May-June) . . . 579*
——Human . . . in leukemia: observations on alveolar capillary leukostasis with reference to pathologic physiology. (*Old, Smith, and Grampa:* May-June) 605*
——Influence of the intensity of exposure upon the number of pulmonary adenomas initiated by a single *in vitro* exposure to ultraviolet irradiation. (*Rogers:* May-June) 582*
——Periarteritis nodosa limited to the pulmonary circulation. (*Braunstein:* September-October) 837
——Pulmonary mucormycosis. (*Baker and Wynn:* May-June) 599*
——Rôle of pulmonary hypertension and thrombo-embolism in the production of pulmonary arteriosclerosis. (*O'Neal and Thomas:* May-June) 578*
——Uremic pneumonitis. (*Hopps and Wissler:* March-April) . . . 261

Luxol fast blue myelin stain—New applications of the . . . . I. A myelo-angio-cyto-architectonic method. II. A myelin-neuroglia method. III. A myelin-fat method. IV. A "myelin"-axis cylinder method. (*Margolis and Pickett:* May-June) 565*

Lymph nodes—Involvement of internal mammary . . . in carcinoma of the breast: studies of the extended radical mastectomy. (*Wyatt, Sugarbaker, and Stanton:* January-February) 143

Lymphoma—On the mechanism of unexpected (so-called spontaneous) rupture of the spleen in malignant . . . . (*Custer, Gunter, Warren, and Collier:* May-June) 597*

Mechanism of unexpected (so-called spontaneous) rupture of the spleen in malignant lymphoma. (*Custer, Gunter, Warren, and Collier:* May-June) 597*

Melanoma—Pigment production and biologic behavior in 380 cases of malignant . . . . (*Russell, Guraieb, Wiltberger, and Clark:* May-June) 585*

Meningococcal toxin—Morphologc changes in rabbits following the intravenous administration of . . . . I. The effects produced in young and in mature animals by a single injection. (*Brunson, Gamble, and Thomas:* May-June) 489

——Morphologic changes in rabbits following the intravenous administration of . . . . II. Two appropriately spaced injections; the rôle of fibrinoid in the generalized Shwartzman reaction. (*Brunson, Thomas, and Gamble:* July-August) 655

——Morphologic changes in rabbits following the intravenous administration of . . . . III. The effects produced by endotoxin in association with certain high molecular weight acidic polymers. (*Brunson, Davis, and Thomas:* July-August) 669

Metachromasia—Factors influencing metachromasy in solutions. (*Schoenberg and Orbison:* May-June) 573*

Methylcholanthrene—Atypical cirrhosis in the duck produced by . . . . (*Rigdon:* May-June) 461

——Experimental carcinogenesis of the skin in mice: dose-response relationships. (*McTurk, Eckardt, Smith, and Cooper:* May-June) . 582*

Mitochondrial changes in hepatic cells of x-irradiated mice. (*MacCardle and Congdon:* July-August) 725

Moniliasis—Experimental . . . in mice. (*Adriano and Schwarz:* September-October) 859

Morphologic changes in rabbits following the intravenous administration of meningococcal toxin. I. The effects produced in young and in mature animals by a single injection. (*Brunson, Gamble, and Thomas:* May-June) 489

——. II. Two appropriately spaced injections; the rôle of fibrinoid in the generalized Shwartzman reaction. (*Brunson, Thomas, and Gamble:* July-August) 655

——. III. The effects produced by endotoxin in association with certain high molecular weight acidic polymers. (*Brunson, Davis, and Thomas:* July-August) 669

Morphologic evidence of the hypersensitive pathogenesis of the lesions of experimental hypersensitivity and the collagen diseases. (*More and Movat:* May-June) 562*

Morphologic evolution of amaurotic family idiocy: the protracted phase of the disease. (*Aronson, Volk, and Epstein:* July-August) 609

Morphologic variation in tissue of the organisms of the blastomycoses and of histoplasmosis. (*Moore:* November-December) 1049

Mortality in relation to histology in Hodgkin's disease. (*Smetana and Cohen:* May-June) 597*

Mouse—Chlorophyllin injection in pregnant mice. (*Glaubach and Antopol:* May-June) 601*

——Experimental carcinogenesis of the skin in mice: dose-response relationships. (*McTurk, Eckardt, Smith, and Cooper:* May-June) . 582*
——Experimental moniliasis in mice. (*Adriano and Schwarz:* September-October) 859
——Histopathologic study of the small intestine of immature non-immunized mice infected with *Trichinella spiralis*. (*Larsh, Jeffries, and Race:* May-June) 561*
——Immunization against Brucella infection. V. Histopathologic appraisal of immunity induced in mice by a streptomycin-dependent mutant of *Brucella melitensis*. (*Elberg, Steiner, and Doll:* November-December) 1065
——LS tumor, a reticulum cell sarcoma of C57BL/6 mice. (*Hathaway and Dowling:* May-June) 583*
——Mitochondrial changes in hepatic cells of x-irradiated mice. (*MacCardle and Congdon:* July-August) 725
——Resistance to quadruple combination chemotherapy in leukemia L1210 in mice. (*Nadel and Hilgar:* May-June) 605*
——Studies on rickettsial toxins. III. Histochemical survey of selected tissue enzymes in mice receiving murine typhus toxin. (*Wattenberg:* September-October) 875
Mucormycosis—Experimental cerebral . . . in diabetic rabbits. (*Bauer, Flanagan, and Sheldon:* May-June) 600*
——Pulmonary . . . . (*Baker and Wynn:* May-June) 599*
Mucoviscidosis—The pathogenesis of fibrocystic disease of the pancreas. Study of the ducts by serial sections. (*Allen and Baggenstoss:* March-April) 337
Mumps encephalitis: pathology and pathogenesis. (*Donohue, Playfair, and Whitaker:* May-June) 565*
Murine typhus—Studies on rickettsial toxins. III. Histochemical survey of selected tissue enzymes in mice receiving . . . toxin. (*Wattenberg:* September-October) 875
Muscle—Lesions of skeletal . . . in leptospirosis. Review of reports and an experimental study. (*Laurain:* May-June) 501
Mycotic aneurysm—Bacterial aortitis and . . . of the aorta: a report of twelve cases. (*Parkhurst and Decker:* September-October) . . . 821
Myelin—New applications of the luxol fast blue . . . stain. I. A myelo-angio-cyto-architectonic method. II. A . . .-neuroglia method. III. A . . .-fat method. IV. A ". . ."-axis cylinder method. (*Margolis and Pickett:* May-June) 565*
Myeloma—Qualitative analyses of antigens of . . . serums with the agar-gel technique. (*Rice:* May-June) 574*
Myoblastoma—Study of cell groups in the hypophysis and their relation to granular cell myoblastomas. (*Davis and Bull:* May-June) . . . 566*
Myocardium—Basophilic degeneration of the . . . . (*Scotti:* May-June) 606*
Myocytolysis—Focal . . . of the heart. (*Schlesinger and Reiner:* May-June) 443

Nature of gliomas as revealed by animal experimentation. (*Zimmerman:* January-February) 1
Necrosis—Adrenal . . . and thrombosis in routine necropsies. (*Plaut:* January-February) 93
Needle biopsy—Correlation of glomerular filtration rate with histopathologic changes in glomeruli as seen in . . . of the kidney. (*Howe and Parrish:* May-June) 603*
——Glomerulonephritis: a correlation of histopathology in needle biopsies of the kidney with the clinical course. (*Howe and Parrish:* May-June) 594*
——Renal diseases as studied by serial kidney biopsy. (*Pirani, Muehrcke, and Kark:* May-June) 594*
Neonatal jaundice with giant cell transformation of the hepatic parenchyma. (*Smetana and Johnson:* July-August) 747

Neoplasia—See also specific neoplasms by name and anatomical sites.
——Association of masculinizing ovarian hilus cell tumors, ovarian stromal hyperplasia, and lutein-like cell proliferation. (*Sternberg:* May-June) 571*
——Carcinolytic action of antibiotics: puromycin and actinomycin D. (*Farber:* May-June) 582*
——Experimental carcinogenesis of the skin in mice: dose-response relationships. (*McTurk, Eckardt, Smith, and Cooper:* May-June) . 582*
——Further studies in quantitative cytopathology: optical measurements of the organic mass and the nucleoprotein mass of chromosomes in normal and neoplastic cells. (*Mellors and Ortega:* May-June) 604*
——Heterotopic ossification in intestinal neoplasms. (*Van Patter and Whittick:* January-February) 73
——Possible endocrine significance of hypophyseal tumors in man. (*Russfield and Reiner:* May-June) 572*
Neoplasm—Neurogenic tumors in the duck: an experimental study. (*Rigdon:* May-June) 583*
——Qualitative analyses of antigens of myeloma serums with the agar-gel technique. (*Rice:* May-June) 574*
——Thymoma: a review and reclassification. (*Iverson:* May-June) . 598*
——Transplantation of the parakeet pituitary tumor. (*Schlumberger:* May-June) 583*
Nephrectomy—Reduction of post-. . . hypertension by homogenous renal transplant mediated by means other than desalting and dehydration. (*Muirhead, Stirman, and Lesch:* May-June) 585*
Nephrosis—The rôle of oxyhemoglobin and its derivatives in the pathogenesis of experimental hemoglobinuric . . . . (*Lalich:* January-February) 153
Nephrotic syndrome induced in rats by aminonucleoside. (*Craig:* May-June) 591*
Neurogenic tumors in the duck: an experimental study. (*Pigdon:* May-June) 583*
New applications of the luxol fast blue myelin stain. I. A myelo-angio-cyto-architectonic method. II. A myelin-neuroglia method. III. A myelin-fat method. IV. A "myelin"-axis cylinder method. (*Margolis and Pickett:* May-June) 565*
New approaches to the study of renal disorders. (*Oliver:* May-June) . 592*
Newcastle disease virus in cultures of chick embryo tissues: its multiplication, titration, and cytopathogenicity. (*Mason and Kaufman:* September-October) 883
Nicotinamide—Pathologic effects of antimetabolites. I. Acute lesions in the hypothalamus, peripheral ganglia, and adrenal medulla caused by 3-acetyl pyridine and prevented by . . . . (*Hicks:* January-February) 189
Nodular and fatty glomerular lesions in rabbits on cortisone. (*Wilens and Stumpf:* March-April) 275
5-Nucleotidase—Histochemical studies on the activation of tissue alkaline phosphatase and . . . . (*Freiman:* May-June) 572*

Ossification—Heterotopic . . . in intestinal neoplasms. (*Van Patter and Whittick:* January-February) 73
Ovary—Association of masculinizing ovarian hilus cell tumors, ovarian stromal hyperplasia, and lutein-like cell proliferation. (*Sternberg:* May-June) 571*
Oxyhemoglobin—The rôle of . . . and its derivatives in the pathogenesis of experimental hemoglobinuric nephrosis. (*Lalich:* January-February) 153

Pancreas—Cytology of islet cells in alloxan diabetic rabbits. (*Bencosme:* November-December) 1149

——Effect of large doses of growth hormone on the pancreatic alpha cells in the rat. (*Volk and Goldner:* May-June) 569*
——Histochemical observations on hyalinized islets of Langerhans in diabetes mellitus. (*Toreson and Rinehart:* May-June) 569*
——Pancreatic lesions and peripancreatic fat necrosis in cortisone-treated rabbits. (*Stumpf and Wilens:* May-June) 563*
——The destructive effects of DL-ethionine on the . . ., stomach, and submaxillary glands. (*Loring and Hartley:* May-June) 521
——The pathogenesis of fibrocystic disease of the . . . . Study of the ducts by serial sections. (*Allen and Baggenstoss:* March-April) . 337
Pancreatic fat necrosis—Pancreatic lesions and peri. . . in cortisone-treated rabbits. (*Stumpf and Wilens:* May-June) 563*
Pancreatic lesions and peripancreatic fat necrosis in cortisone-treated rabbits. (*Stumpf and Wilens:* May-June) 563*
Parakeet—Transplantation of the . . . pituitary tumor. (*Schlumberger:* May-June) 583*
Pathogenesis of bovine ocular squamous cell carcinoma: histopathologic and clinical studies. (*Wynne and Russell:* May-June) 584*
Pathogenesis of death due to burning of the skin. (*Rosenthal, Finamore, Hunter, Hunter, and Roman:* May-June) 568*
Pathogenesis of fibrocystic disease of the pancreas. Study of the ducts by serial sections. (*Allen and Baggenstoss:* March-April) . . . 337
Pathogenetic studies in the experimental hepatorenal syndrome using micro-angiography in the agonal phase. (*Crowson, More, and King:* May-June) 593*
Pathologic and physiologic effects of hemorrhagic shock on the heart. (*Hackel:* May-June) 576*
Pathologic effects of antimetabolites. I. Acute lesions in the hypothalamus, peripheral ganglia, and adrenal medulla caused by 3-acetyl pyridine and prevented by nicotinamide. (*Hicks:* January-February) 189
Pathologic findings associated with unexpected death in infants and children. (*Arey:* May-June) 578*
Pathology of hemorrhagic fever. A comparison of the findings — 1951 and 1952. (*Steer:* March-April) 201
Pathology of infectious serositis of ducks. (*Dougherty, Saunders, and Parsons:* May-June) 475
Pathology of poliomyelitis in the chick embryo. (*Love and Roca-Garcia:* September-October) 901
Pathology of swine exposed to total body gamma radiation from an atomic bomb source. (*Tullis, Lamson, and Madden:* January-February) 41
Pathophysiologic evaluation in dogs of two surgical procedures used in patients with occlusive coronary artery disease. (*Maniglia and Bakst:* May-June) 574*
Pattern of organ infiltrate in acute leukemia of children. (*Cochran and Haut:* May-June) 597*
Periarteritis nodosa limited to the pulmonary circulation. (*Braunstein:* September-October) 837
Phosphatase—Histochemical studies on the activation of tissue alkaline . . . and 5-nucleotidase. (*Freiman:* May-June) 572*
Pigment production and biologic behavior in 380 cases of malignant melanoma. (*Russell, Guraieb, Wiltberger, and Clark:* May-June) . 585*
Pituitary body—See hypophysis.
Pneumonia—Experimental aspiration . . .: inflammatory and reparative changes produced by intratracheal injections of autologous gastric juice and hydrochloric acid. (*Moran:* May-June) . . . 579*
Pneumonitis—Granulomatous . . . caused by short-fibered asbestos dust (chrysotile). (*Gross, Westrick, and McNerney:* May-June) . . . 579*
——Uremic . . . . (*Hopps and Wissler:* March-April) 261

Poliomyelitis—Pathology of . . . in the chick embryo. (*Love and Roca-Garcia:* September-October) 901
Possible endocrine significance of hypophyseal tumors in man. (*Russfield and Reiner:* May-June) 572*
Potassium—Sodium and . . . content of rabbit plasma and erythrocytes in relation to age and to blood pressure. (*Waugh and Stuart:* May-June) 575*
Proceedings—See American Association of Pathologists and Bacteriologists.
Prolonged functional survival of a renal homotransplant in man: morphologic observations with clinical correlation. (*Dammin, Hume, Merrill, Miller, and Thorn:* May-June) 586*
Pseudolymphocytic choriomeningitis—The viral range *in vitro* of a malignant human epithelial cell (strain HeLa, Gey). III. Studies with . . . virus; general discussion. (*Scherer and Syverton:* January-February) 31
Pulmonary mucormycosis. (*Baker and Wynn:* May-June) 599*
Puromycin—Carcinolytic action of antibiotics: . . . and actinomycin D. (*Farber:* May-June) 582*

Qualitative analyses of antigens of myeloma serums with the agar-gel technique. (*Rice:* May-June) 574*

Rabbit—Cytology of islet cells in alloxan diabetic rabbits. (*Bencosme:* November-December) 1149
——Experimental cerebral mucormycosis in diabetic rabbits. (*Bauer, Flanagan, and Sheldon:* May-June) 600*
——Glomerular lesions and the nephrotic syndrome in rabbits given saccharated iron oxide intravenously. (*Ellis:* May-June) . . . 591*
——Intimal repair of the aorta of the . . . following experimental trauma. (*Prior and Hutter:* January-February) 107
——Morphologic changes in rabbits following the intravenous administration of meningococcal toxin. I. The effects produced in young and in mature animals by a single injection. (*Brunson, Gamble, and Thomas:* May-June) 489
——Morphologic changes in rabbits following the intravenous administration of meningococcal toxin. II. Two appropriately spaced injections; the rôle of fibrinoid in the generalized Shwartzman reaction. (*Brunson, Thomas, and Gamble:* July-August) 655
——Morphologic changes in rabbits following the intravenous administration of meningococcal toxin. III. The effects produced by endotoxin in association with certain high molecular weight acidic polymers. (*Brunson, Davis, and Thomas:* July-August) . . . 669
——Nodular and fatty glomerular lesions in rabbits on cortisone. (*Wilens and Stumpf:* March-April) 275
——Pancreatic lesions and peripancreatic fat necrosis in cortisone-treated rabbits. (*Stumpf and Wilens:* May-June) 563*
——Sodium and potassium content of . . . plasma and erythrocytes in relation to age and to blood pressure. (*Waugh and Stuart:* May-June) 575*
Rat—A case of leukemic reticulo-endotheliosis in the . . . . The relationship between this entity and Naegeli's monocytic myelogenous leukemia. (*Harris, Shay, and Gruenstein:* March-April) 367
——Effect of large doses of growth hormone on the pancreatic alpha cells in the . . . . (*Volk and Goldner:* May-June) 569*
——Effects of massive whole body radiation in rats. (*Koletsky:* May-June) 568*
——Experimental atherosclerosis in the . . . . (*Bragdon and Mickelsen:* September-October) 965
——Fat emboli in glomerular capillaries of choline-deficient rats and of patients with diabetic glomerulosclerosis. (*Hartroft:* May-June) . 381

——Histochemical localization of certain oxidative enzymes during the production of infarcts of the . . . kidney. (*Peters and Farber:* May-June) 590*
——Increased spontaneous occurrence of fibroadenomas of the breast in the albino . . . influenced by a high fat diet. (*Benson, Lev, and Grand:* May-June) 583*
——Influence of cortisone on the development of the experimental silica granuloma in the . . . . (*Clark:* May-June) 563*
——Nephrotic syndrome induced in rats by aminonucleoside. (*Craig:* May-June) 591*
——Susceptibility of endocardium of altitude rats to various bacteria. (*Highman and Allland:* May-June) 603*
Reduction of post-nephrectomy hypertension by homogenous renal transplant mediated by means other than desalting and dehydration. (*Muirhead, Stirman, and Lesch:* May-June) 585*
Relapsing fever in Korea: a clinicopathologic study of eleven fatal cases with special attention to association with Salmonella infections. (*Anderson and Zimmerman:* November-December) 1083
Relation of splenic calcification to histoplasmosis. (*Schwarz, Silverman, Straub, Levine, and Adriano:* May-June) 606*
Renal diseases as studied by serial kidney biopsy. (*Pirani, Muehrcke, and Kark:* May-June) 594*
Renal Shwartzman phenomenon in trichinosis. (*Yesner:* May-June) . 592*
Resistance to quadruple combination chemotherapy in leukemia L1210 in mice. (*Nadel and Hilgar:* May-June) 605*
Reticulo-endotheliosis—A case of leukemic . . . in the rat. The relationship between this entity and Naegeli's monocytic myelogenous leukemia. (*Harris, Shay, and Gruenstein:* March-April) 367
Retrocardiac, intercoronary, and transpleural components in experimentally produced collateral circulation to the heart. (*Kline, Bloomer, Stern, and Liebow:* May-June) 574*
Rheumatic activity in auricular appendages removed at mitral valvoplasty. (*Clark and Anderson:* September-October) 809
Rheumatic carditis—Clinical significance of the Aschoff body based on morphologic and histochemical observations. (*Tedeschi, Wagner, and Pani:* May-June) 576*
Rickettsia—Studies on rickettsial toxins. III. Histochemical survey of selected tissue enzymes in mice receiving murine typhus toxin. (*Wattenberg:* September-October) 875
Rôle of oxyhemoglobin and its derivatives in the pathogenesis of experimental hemoglobinuric nephrosis. (*Lalich:* January-February) . 153
Rôle of pulmonary hypertension and thrombo-embolism in the production of pulmonary arteriosclerosis. (*O'Neal and Thomas:* May-June) 578*
Saccharated iron oxide—Glomerular lesions and the nephrotic syndrome in rabbits given . . . intravenously. (*Ellis:* May-June) 591*
Salmonella—Relapsing fever in Korea: a clinicopathologic study of eleven fatal cases with special attention to association with . . . infections. (*Anderson and Zimmerman:* November-December) . . 1083
Sarcoma—LS tumor, a reticulum cell . . . of C57BL/6 mice. (*Hathaway and Dowling:* May-June) 583*
Sarcoma arising in glioblastoma of the brain. (*Feigin and Gross:* July-August) 633
Schistosomiasis—Lesions of the intrahepatic portal radicles in Manson's . . . . (*Lichtenberg:* May-June, 604*; July-August) 757
Schultz-Dale test—Detection of soluble carcinoma antigen by use of the . . . . (*Makari:* May-June) 581*
Selective renal vasospasm and ischemic renal necrosis produced experimentally with staphylococcal toxin. Observations on the pathogenesis of bilateral cortical necrosis. (*Thal:* March-April) . . . 233

Serositis—The pathology of infectious . . . of ducks. (*Dougherty, Saunders, and Parsons:* May-June) 475
Serum elpherograms in experimental nephropathies of guinea-pigs. (*Pollak and Cruz:* May-June) 573*
Shock—Pathologic and physiologic effects of hemorrhagic . . . on the heart. (*Hackel:* May-June) 576*
Shwartzman phenomenon—Local renal . . . . (*Black-Schaffer and Garcia-Caceres:* May-June) 592*
——Morphologic changes in rabbits following the intravenous administration of meningococcal toxin. II. Two appropriately spaced injections; the rôle of fibrinoid in the generalized Shwartzman reaction. (*Brunson, Thomas, and Gamble:* July-August) 655
——Renal . . . in trichinosis. (*Yesner:* May-June) 592*
Signet-ring cell carcinoma of the urinary bladder. (*Saphir:* March-April) 223
Silicosis—Influence of cortisone on the development of the experimental silica granuloma in the rat. (*Clark:* May-June) 563*
Skin—On the pathogenesis of death due to burning of the . . . . (*Rosenthal, Finamore, Hunter, Hunter, and Roman:* May-June) 568*
Sodium and potassium content of rabbit plasma and erythrocytes in relation to age and to blood pressure. (*Waugh and Stuart:* May-June) 575*
Sound—Effect of audiogenic stimuli on wound healing in DBA/1 mice. (*Spain, Molomut, and Riess:* May-June) 607*
Spleen—On the mechanism of unexpected (so-called spontaneous) rupture of the . . . in malignant lymphoma. (*Custer, Gunter, Warren, and Collier:* May-June) 597*
——Relation of splenic calcification to histoplasmosis. (*Schwarz, Silverman, Straub, Levine, and Adriano:* May-June) 606*
Squamous epithelium—Comparison of histochemical properties of normal and neoplastic . . . . (*Foraker:* May-June) 581*
Staphylococcal toxin—Selective renal vasospasm and ischemic renal necrosis produced experimentally with . . . . Observations on the pathogenesis of bilateral cortical necrosis. (*Thal:* March-April) . . 233
Stomach—The destructive effects of DL-ethionine on the pancreas, . . ., and submaxillary glands. (*Loring and Hartley:* May-June) . . . 521
Strain of metastatic epithelial cells derived from long-term tissue culture of human bone marrow. (*Berman, Stulberg, and Ruddle:* May-June) 580*
Structural features of goiters in sporadic cretins. (*Milles:* November-December) 997
Studies on hypervitaminosis D. (*Follis:* May-June) 568*
Studies on rickettsial toxins. III. Histochemical survey of selected tissue enzymes in mice receiving murine typhus toxin. (*Wattenberg:* September-October) 875
Studies on Trichinella spiralis. I to V. (*Gould, Gomberg, Bethell, Villella, and Hertz:* September-October) 933
Study of cell groups in the hypophysis and their relation to granular cell myoblastomas. (*Davis and Butt:* May-June) 566*
Submaxillary glands—The destructive effects of DL-ethionine on the pancreas, stomach, and . . . . (*Loring and Hartley:* May-June) . 521
Succinic dehydrogenase activity in myocardial infarction and in induced myocardial necrosis. (*Wachstein and Meisel:* March-April) . . . 353
Susceptibility in vitro of adult horse tissue to equine abortion virus: previously undescribed lesions. (*Randall:* November-December) . 1165
Susceptibility of endocardium of altitude rats to various bacteria. (*Highman and Altland:* May-June) 603*
Swine—Pathology of . . . exposed to total body gamma radiation from an atomic bomb source. (*Tullis, Lamson, and Madden:* January-February) 41
Symposium on new approaches to the study of renal disorders. (*Oliver:* May-June) 585*

Systemic effects of carbon particles coated with antigen. (*Garcia-Caceres and Black-Schaffer:* May-June) 601*
Systemic karyomegaly. Case report of a heretofore undescribed disease entity. (*Moritz and Leuchtenberger:* May-June) 564*

Thermal injury—On the pathogenesis of death due to burning of the skin. (*Rosenthal, Finamore, Hunter, Hunter, and Roman:* May-June) . . 568*
Three-dimensional analysis of hepatocellular carcinoma. (*Elias and Popper:* May-June) 596*
Thrombosis—Adrenal necrosis and . . . in routine necropsies. (*Plaut:* January-February) 93
Thymoma: a review and reclassification. (*Iverson:* May-June) 598*
Thyroid gland—Structural features of goiters in sporadic cretins. (*Milles:* November-December) 997
Tissue culture of human breast carcinoma. (*Orr and McSwain:* January-February) 125
Toxin—Studies on rickettsial toxins. III. Histochemical survey of selected tissue enzymes in mice receiving murine typhus . . . . (*Wattenberg:* September-October) 875
Transplantation—Prolonged functional survival of a renal homotransplant in man: morphologic observations with clinical correlation. (*Dammin, Hume, Merrill, Miller, and Thorn:* May-June) 586*
——Reduction of post-nephrectomy hypertension by homogenous renal transplant mediated by means other than desalting and dehydration. (*Muirhead, Stirman, and Lesch:* May-June) 585*
Transplantation of the parakeet pituitary tumor. (*Schlumberger:* May-June) 583*
Trauma—Intimal repair of the aorta of the rabbit following experimental . . . . (*Prior and Hutter:* January-February) 107
——Lesions of the corpus callosum following blunt mechanical . . . to the head. (*Lindenberg, Fisher, Durlacher, Lovitt, and Freytag:* March-April) 297
Trichinella spiralis—Effect of feeding irradiated trichinella larvae on production of immunity to re-infection. (*Gould, Gomberg, Bethell, Villella, and Hertz:* May-June) 561*
——Histopathologic study of the small intestine of immature non-immunized mice infected with . . . . (*Larsh, Jeffries, and Race:* May-June) 561*
——Studies on . . . . I to V. (*Gould, Gomberg, Bethell, Villella, and Hertz:* September-October) 933
Trichinosis—Renal Shwartzman phenomenon in . . . . (*Yesner:* May-June) 592*
Tryptophan—Histopathology of amino acid deficiencies. IV. . . . . (*Scott:* November-December) 1111
Tubercle bacillus—Bacterial endocarditis produced by the . . . .(*Russell and Berthrong:* May-June) 577*
Tuberculosis—Cystic disease of the lungs: a consequence of the healing of acute disseminated pulmonary . . . . (*Berthrong and Ganem:* May-June) 601*

Ultraviolet absorption—Glomerular ultraviolet absorptions after cortisone therapy, simulating diabetes mellitus. (*Sommers:* May-June) . 570*
Unexpected death—Pathologic findings associated with . . . in infants and children: (*Arey:* May-June) 578*
Uremic pneumonitis. (*Hopps and Wissler:* March-April) 261
Urinary bladder—Signet-ring cell carcinoma of the . . . . (*Saphir:* March-April) 223
Uterus—Cancer of the cervix uteri: a review of 169 necropsied cases. (*Marcial-Rojas and Meigs:* November-December) 1077

Viral range in vitro of a malignant human epithelial cell (strain HeLa, Gey). III. Studies with pseudolymphocytic choriomeningitis virus; general discussion. (*Scherer and Syverton:* January-February) . . 31
Virus—Effect of hyaluronidase antagonists on the reaction of the host to viral infection. (*Pearce:* May-June) 562*
——Newcastle disease . . . in cultures of chick embryo tissues: its multiplication, titration, and cytopathogenicity. (*Mason and Kaufman:* September-October) 883
——Pathology of poliomyelitis in the chick embryo. (*Love and Roca-Garcia:* September-October) 901
——Susceptibility *in vitro* of adult horse tissue to equine abortion . . .: previously undescribed lesions. (*Randall:* November-December) . 1165
——The viral range *in vitro* of a malignant human epithelial cell (strain HeLa, Gey). III. Studies with pseudolymphocytic choriomeningitis . . .; general discussion. (*Scherer and Syverton:* January-February) 31
Vitamin D—Studies on hypervitaminosis D. (*Follis:* May-June) . . . 568*

Wilson's disease—Histochemical demonstration of copper in a case of hepatolenticular degeneration. (*Green:* May-June) 545
Wound healing—Effect of audiogenic stimuli on . . . in DBA/1 mice. (*Spain, Molomut, and Riess:* May-June) 607*

INDEX OF AUTHORS

Adriano, S. See Schwarz, Silverman, Straub, Levine, and Adriano (May-June) 606*

——, **and Schwarz, J.** Experimental moniliasis in mice. (September-October) 859

Allan, J. S. See Hales, Allan, and Hall (May-June) 595*

Allen, R. A., and Baggenstoss, A. H. The pathogenesis of fibrocystic disease of the pancreas. Study of the ducts by serial sections. (March-April) 337

Altland, P. D. See Highman and Altland (May-June) 603*

Anderson, T. R., and Zimmerman, L. E. Relapsing fever in Korea: a clinicopathologic study of eleven fatal cases with special attention to association with Salmonella infections. (November-December) . . 1083

Anderson, W. See Clark and Anderson (September-October) . . . 809

Antopol, W. See Glaubach and Antopol (May-June) 601*

Arey, J. B. Pathologic findings associated with unexpected death in infants and children. (May-June) 578*

Arias-Stella, J. See Mellors, Arias-Stella, Siegel, and Pressman (July-August) 687

Aronson, S. M.; Volk, B. W., and Epstein, N. Morphologic evolution of amaurotic family idiocy: the protracted phase of the disease. (July-August) 609

Baggenstoss, A. H. See Allen and Baggenstoss (March-April) . . . 337

Baker, R. D., and Wynn, J. O. Pulmonary mucormycosis. (May-June) . 599*

Bakst, A. A. See Maniglia and Bakst (May-June) 574*

Bauer, H.; Flanagan, J. F., and Sheldon, W. H. Experimental cerebral mucormycosis in diabetic rabbits. (May-June) 600*

Bencosme, S. A. Cytology of islet cells in alloxan diabetic rabbits. (November-December) 1149

Benson, J.; Lev, M., and Grand, C. G. Increased spontaneous occurrence of fibroadenomas of the breast in the albino rat influenced by a high fat diet. (May-June) 583*

Berman, L.; Stulberg, C. S., and Ruddle, F. A strain of metastatic epithelial cells derived from long-term tissue culture of human bone marrow. (May-June) 580*

Berthrong, M. See Russell and Berthrong (May-June) 577*

——, **and Ganem, G.** Cystic disease of the lungs: a consequence of the healing of acute disseminated pulmonary tuberculosis. (May-June) 601*

Bethell, F. H. See Gould, Gomberg, Bethell, Villella, and Hertz (May-June, 561*; September-October) 933

Black-Schaffer, B. See Garcia-Caceres and Black-Schaffer (May-June) . 601*

——, **and Garcia-Caceres, U.** Local renal Shwartzman phenomenon. (May-June) 592*

Bloodworth, J. M. B., Jr., and Hamwi, G. J. Histopathology of experimental glomerular lesions simulating human diabetic glomerulosclerosis. (January-February) 167

Bloomer, W. E. See Kline, Bloomer, Stern, and Liebow (May-June) . . 574*

Bragdon, J. H., and Mickelsen, O. Experimental atherosclerosis in the rat. (September-October) 965

Braunstein, H. Periarteritis nodosa limited to the pulmonary circulation. (September-October) 837

Brindley, P., and Stembridge, V. A. Aneurysms of the aorta: a report of 358 cases. (May-June) 576*

Brinkhous, K. M. See Swanton and Brinkhous (May-June) 596*

Bruce, C. See Popper, Clearkin, Kent, Parthasarathy, and Bruce (May-June) 594*

* Abstract of paper presented at the meeting of the American Association of Pathologists and Bacteriologists held at Houston, Texas, April 7, 8, and 9, 1955.

Brunson, J. G.; Davis, R. L., and Thomas, L. Morphologic changes in rabbits following the intravenous administration of meningococcal toxin. III. The effects produced by endotoxin in association with certain high molecular weight acidic polymers. (July-August) . . 669
——; Gamble, C. N., and Thomas, L. Morphologic changes in rabbits following the intravenous administration of meningococcal toxin. I. The effects produced in young and in mature animals by a single injection. (May-June) 489
——; Thomas, L., and Gamble, C. N. Morphologic changes in rabbits following the intravenous administration of meningococcal toxin. II. Two appropriately spaced injections; the rôle of fibrinoid in the generalized Shwartzman reaction. (July-August) 655
Butt, E. M. See Davis and Butt (May-June) 566*

Castleman, B. See Cohen, Chapman, and Castleman (May-June) . . 570*
Chapman, W. See Cohen, Chapman, and Castleman (May-June) . . 570*
Churg, J. See Grishman and Churg (May-June) 602*
Clark, R. L., Jr. See Russell, Guraieb, Wiltberger, and Clark (May-June) 585*
Clark, R. M., and Anderson, W. Rheumatic activity in auricular appendages removed at mitral valvoplasty. (September-October) . . . 809
Clark, W. H., Jr. Influence of cortisone on the development of the experimental silica granuloma in the rat. (May-June) 563*
Clearkin, K. See Popper, Clearkin, Kent, Parthasarathy, and Bruce (May-June) 594*
Cochran, T. H., and Haut, A. Pattern of organ infiltrate in acute leukemia of children. (May-June) 597*
Cohen, B. M. See Smetana and Cohen (May-June) 597*
Cohen, R. B.; Chapman, W., and Castleman, B. Hyperadrenocorticism: a histochemical study of surgically resected adrenal glands. (May-June) 570*
Collier, F. C. See Custer, Gunter, Warren, and Collier (May-June) . . 597*
Congdon, C. C. See MacCardle and Congdon (July-August) 725
Cooper, N. S. See McTurk, Eckardt, Smith, and Cooper (May-June) . 582*
Craig, J. M. Nephrotic syndrome induced in rats by aminonucleoside. (May-June) 591*
Crowson, C. N.; More, R. H., and King, J. B. Pathogenetic studies in the experimental hepatorenal syndrome using micro-angiography in the agonal phase. (May-June) 593*
Cruz, P. T. See Pollak and Cruz (May-June) 573*
Custer, R. P.; Gunter, M. J.; Warren, S., and Collier, F. C. On the mechanism of unexpected (so-called spontaneous) rupture of the spleen in malignant lymphoma. (May-June) 597*

Dammin, G. J.; Goldman, M. L.; Schroeder, H. A., and Pace, M. G. Effects of prolonged experimental hypertension in the dog with a study of periodic renal biopsies over a seven-year period. (May-June) 587*
——; Hume, D. M.; Merrill, J. P.; Miller, B. F., and Thorn, G. W. Prolonged functional survival of a renal homotransplant in man: morphologic observations with clinical correlation. (May-June) . . 586*
Davis, F. E., and Butt, E. M. Study of cell groups in the hypophysis and their relation to granular cell myoblastomas. (May-June) . . . 566*
Davis, O. F. See Oester, Davis, and Friedman (July-August) 717
Davis, R. L. See Brunson, Davis, and Thomas (July-August) . . . 669
Decker, J. P. See Parkhurst and Decker (September-October) . . . 821
Diamond, I. Kwashiorkor in a white American male. (May-June) . . 596*
Doll, J. P. See Elberg, Steiner, and Doll (November-December) . . 1065
Donohue, W. L.; Playfair, F. D., and Whitaker, L. Mumps encephalitis: pathology and pathogenesis. (May-June) 565*
Dougherty, E., 3rd; Saunders, L. Z., and Parsons, E. H., Jr. The pathology of infectious serositis of ducks. (May-June) 475

Dowling, E. A. See Hathaway and Dowling (May-June) 583*
Durlacher, S. H. See Lindenberg, Fisher, Durlacher, Lovitt, and Freytag (March-April) 297

Eckardt, R. E. See McTurk, Eckardt, Smith, and Cooper (May-June) . 582*
Ehrlich, J. C., and Ratner, I. M. Congenital cirrhosis of the liver with kernicterus: report of two cases in siblings with a discussion of the relationship to so-called neonatal hepatitis and to iso-immunization disease. (November-December) 1013
Elberg, S. S.; Steiner, P. E., and Doll, J. P. Immunization against Brucella infection. V. Histopathologic appraisal of immunity induced in mice by a streptomycin-dependent mutant of *Brucella melitensis*. (November-December) 1065
Elias, H. See Popper and Elias (May-June) 405
——, and Popper, H. Three-dimensional analysis of hepatocellular carcinoma. (May-June) 596*
Ellis, J. T. Glomerular lesions and the nephrotic syndrome in rabbits given saccharated iron oxide intravenously. (May-June) . . . 591*
Epstein, N. See Aronson, Volk, and Epstein (July-August) 609

Farber, E. Improved method for the histochemical localization of oxidative enzymes in the kidney with tetrazolium. (May-June) . . 589*
——. See Peters and Farber (May-June) 590*
Farber, S. Carcinolytic action of antibiotics: puromycin and actinomycin D. (May-June) 582*
Farquhar, M. G. See Rinehart, Farquhar, and Gould (May-June) . . 587*
Feigin, I. H., and Gross, S. W. Sarcoma arising in glioblastoma of the brain. (July-August) 633
Finamore, F. See Rosenthal, Finamore, Hunter, Hunter, and Roman (May-June) 568*
Fisher, R. S. See Lindenberg, Fisher, Durlacher, Lovitt, and Freytag (March-April) 297
Flanagan, J. F. See Bauer, Flanagan, and Sheldon (May-June) . . . 600*
Follis, R. H., Jr. Studies on hypervitaminosis D. (May-June) . . . 568*
Foraker, A. G. Comparison of histochemical properties of normal and neoplastic squamous epithelium. (May-June) 581*
Freiman, D. G. Histochemical studies on the activation of tissue alkaline phosphatase and 5-nucleotidase. (May-June) 572*
Freytag, E. See Lindenberg, Fisher, Durlacher, Lovitt, and Freytag (March-April) 297
Friedman, B. See Oester, Davis, and Friedman (July-August) . . . 717

Gamble, C. N. See Brunson, Gamble, and Thomas (May-June) . . . 489
——. See Brunson, Thomas, and Gamble (July-August) 655
Ganem, G. See Berthrong and Ganem (May-June) 601*
Garcia-Caceres, U. See Black-Schaffer and Garcia-Caceres (May-June) 592*
——, and Black-Schaffer, B. Systemic effects of carbon particles coated with antigen. (May-June) 601*
Gatzimos, C. D.; Schulz, D. M., and Newnum, R. L. Cystinosis (Lignac-Fanconi disease). (May-June, 567*; September-October) . . . 791
Glaubach, S., and Antopol, W. Chlorophyllin injection in pregnant mice. (May-June) 601*
Goldman, M. L. See Dammin, Goldman, Schroeder, and Pace (May-June) 587*
Goldner, M. G. See Volk and Goldner (May-June) 569*
Gomberg, H. J. See Gould, Gomberg, Bethell, Villella, and Hertz (May-June, 561*; September-October) 933
Gould, E. See Rinehart, Farquhar, and Gould (May-June) 587*
Gould, S. E.; Gomberg, H. J.; Bethell, F. H.; Villella, J. B., and Hertz, C. S. Effect of feeding irradiated trichinella larvae on production of immunity to re-infection. (May-June) 561*

——; ——; ——; ——, and ——. Studies on *Trichinella spiralis*. I to V. (September-October) 933
Grampa, G. See Old, Smith, and Grampa (May-June) 605*
Grand, C. G. See Benson, Lev, and Grand (May-June) 583*
Green, C. L. Histochemical demonstration of copper in a case of hepatolenticular degeneration. (May-June) 545
Grishman, E., and Churg, J. Application of thin sections to the study of glomerular changes in glomerulonephritis. (May-June) 602*
Gross, P.; Westrick, M. L., and McNerney, J. M. Granulomatous pneumonitis caused by short-fibered asbestos dust (chrysotile). (May-June) 579*
Gross, S. W. See Feigin and Gross (July-August) 633
Gruenstein, M. See Harris, Shay, and Gruenstein (March-April) . . . 367
Gunter, M. J. See Custer, Gunter, Warren, and Collier (May-June) . . 597*
Guraieb, S. See Russell, Guraieb, Wiltberger, and Clark (May-June) . 585*

Hackel, D. B. Pathologic and physiologic effects of nemorrhagic shock on the heart. (May-June) 576*
Hales, M. R.; Allan, J. S., and Hall, E. M. Injection corrosion studies of the vascular systems of normal and cirrhotic livers. (May-June) . 595*
Hall, E. M. See Hales, Allan, and Hall (May-June) 595*
Hamwi, G. J. See Bloodworth and Hamwi (January-February) . . . 167
Harris, C.; Shay, H., and Gruenstein, M. A case of leukemic reticuloendotheliosis in the rat. The relationship between this entity and Naegeli's monocytic myelogenous leukemia. (March-April) . . . 367
Hartley, L. J. See Loring and Hartley (May-June) 521
Hartroft, W. S. Fat emboli in glomerular capillaries of choline-deficient rats and of patients with diabetic glomerulosclerosis. (May-June) . 381
Hathaway, C. O., and Dowling, E. A. LS tumor, a reticulum cell sarcoma of C57BL/6 mice. (May-June) 583*
Haut, A. See Cochran and Haut (May-June) 597*
Hertz, C. S. See Gould, Gomberg, Bethell, Villelia, and Hertz (May-June, 561*; September-October) 933
Hicks, S. P. Pathologic effects of antimetabolites. I. Acute lesions in the hypothalamus, peripheral ganglia, and adrenal medulla caused by 3-acetyl pyridine and prevented by nicotinamide. (January-February) 189
Highman, B., and Atland, P. D. Susceptibility of endocardium of altitude rats to various bacteria. (May-June) 603*
Hilgar, A. G. See Nadel and Hilgar (May-June) 605*
Hopps, H. C., and Wissler, R. W. Uremic pneumonitis. (March-April) 261
Howe, J. S., and Parrish, A. E. Correlation of glomerular filtration rate with histopathologic changes in glomeruli as seen in needle biopsy of the kidney. (May-June) 603*
——, and ——. Glomerulonephritis: a correlation of histopathology in needle biopsies of the kidney with the clinical course. (May-June) . 594*
Hume, D. M. See Dammin, Hume, Merrill, Miller, and Thorn (May-June) 586*
Hunter, A. S. See Rosenthal, Finamore, Hunter, Hunter, and Roman (May-June) 568*
Hunter, F. R. See Rosenthal, Finamore, Hunter, Hunter, and Roman (May-June) 568*
Hutter, R. V. P. See Prior and Hutter (January-February) 107

Iverson, L. Thymoma: a review and reclassification. (May-June) . . 598*

Jeffries, W. B. See Larsh, Jeffries, and Race (May-June) 561*
Johnson, F. B. See Smetana and Johnson (July-August) 747

Kark, R. M. See Pirani, Muehrcke, and Kark (May-June) 594*
Kaufman, N. See Mason and Kaufman (September-October) . . . 883

Kent, G. See Popper, Clearkin, Kent, Parthasarathy, and Bruce (May-June) 594*
Kent, S. Fat embolism in diabetic patients without physical trauma. (May-June) 399
King, J. B. See Crowson, More, and King (May-June) 593*
Kline, J. L.; Bloomer, W. E.; Stern, H., and Liebow, A. A. Retrocardiac, intercoronary, and transpleural components in experimentally produced collateral circulation to the heart. (May-June) 574*
Koletsky, S. Effects of massive whole body radiation in rats. (May-June) 568*

Laipply, T. C., and Sherrick, J. C. Intrathoracic angiomyomatous hyperplasia associated with chronic chylothorax. (May-June) 599*
Lalich, J. J. The rôle of oxyhemoglobin and its derivatives in the pathogenesis of experimental hemoglobinuric nephrosis. (January-February) 153
Lamson, B. G. See Tullis, Lamson, and Madden (January-February) . 41
Larsh, J. E., Jr.; Jeffries, W. B., and Race, G. J. Histopathologic study of the small intestine of immature non-immunized mice infected with *Trichinella spiralis*. (May-June) 561*
Laurain, A. R. Lesions of skeletal muscle in leptospirosis. Review of reports and an experimental study. (May-June) 501
Lesch, W. See Muirhead, Stirman, and Lesch (May-June) 585*
Leuchtenberger, C. See Moritz and Leuchtenberger (May-June) . . 564*
Lev, M. See Benson, Lev, and Grand (May-June) 583*
Levine, S. See Schwarz, Silverman, Straub, Levine, and Adriano (May-June) 606*
Lichtenberg, F. Lesions of the intrahepatic portal radicles in Manson's schistosomiasis. (May-June, 604*; July-August) 757
Liebow, A. A. See Kline, Bloomer, Stern, and Liebow (May-June) . . 574*
Lindenberg, R.; Fisher, R. S.; Durlacher, S. H.; Lovitt, W. V., Jr., and Freytag, E. Lesions of the corpus callosum following blunt mechanical trauma to the head. (March-April) 297
Loring, W. E., and Hartley, L. J. The destructive effects of DL-ethionine on the pancreas, stomach, and submaxillary glands. (May-June) . 521
Love, R., and Roca-Garcia, M. Pathology of poliomyelitis in the chick embryo. (September-October) 901
Lovitt, W. V., Jr. See Lindenberg, Fisher, Durlacher, Lovitt, and Freytag (March-April) 297
Lux, J. J. See Shapiro, Lux, and Sprofkin (March-April) 319

MacCardle, R. C., and Congdon, C. C. Mitochondrial changes in hepatic cells of x-irradiated mice. (July-August) 725
Madden, S. C. See Tullis, Lamson, and Madden (January-February) . 41
Makari, J. G. Detection of soluble carcinoma antigen by use of the Schultz-Dale test. (May-June) 581*
Maniglia, R., and Bakst, A. A. Pathophysiologic evaluation in dogs of two surgical procedures used in patients with occlusive coronary artery disease. (May-June) 574*
Marcial-Rojas, R. A., and Meigs, J. V. Cancer of the cervix uteri: a review of 169 necropsied cases. (November-December) 1077
Margolis, G., and Pickett, J. P. New applications of the luxol fast blue myelin stain. I. A myelo-angio-cyto-architectonic method. II. A myelin-neuroglia method. III. A myelin-fat method. IV. A "myelin"-axis cylinder method. (May-June) 565*
Mason, E. J., and Kaufman, N. Newcastle disease virus in cultures of chick embryo tissues: its multiplication, titration, and cytopathogenicity. (September-October) 883
McManus, J. F. A. Ischemic glomeruli and their significance for glomerular structure. (May-June) 589*
McMillan, J. B. Embolization of cerebral tissue to lungs following severe head injury. (May-June) 579*

McNerney, J. M. See Gross, Westrick, and McNerney (May-June) . 579*
McSwain, B. See Orr and McSwain (January-February) 125
McTurk, L. C.; Eckardt, R. E.; Smith, W. E., and Cooper, N. S. Experimental carcinogenesis of the skin in mice: dose-response relationships. (May-June) 582*
Meigs, J. V. See Marcial-Rojas and Meigs (November-December) . . 1077
Meisel, E. See Wachstein and Meisel (March-April, 353; May-June) . 588*
Meissner, W. A., and Sommers, S. C. Endometrial hyperplasia and carcinoma produced by estrogen. (May-June) 571*
Mellors, R. C.; Arias-Stella, J.; Siegel, M., and Pressman, D. Analytical pathology. II. Histopathologic demonstration of glomerular-localizing antibodies in experimental glomerulonephritis. (May-June, 592*; July-August) 687
——, **and Ortega, L. G.** Further studies in quantitative cytopathology: optical measurements of the organic mass and the nucleoprotein mass of chromosomes in normal and neoplastic cells. (May-June) . 604*
Merrill, J. P. See Dammin, Hume, Merrill, Miller, and Thorn (May-June) 586*
Michael, M., Jr. See Vogel, Michael, and Timpe (May-June) . . . 535
Mickelsen, O. See Bragdon and Mickelsen (September-October) . . . 965
Miller, B. F. See Dammin, Hume, Merrill, Miller, and Thorn (May-June) 586*
Milles, G. Structural features of goiters in sporadic cretins. (November-December) 997
Molomut, N. See Spain, Molomut, and Riess (May-June) 607*
Moore, M. Morphologic variation in tissue of the organisms of the blastomycoses and of histoplasmosis. (November-December) . . . 1049
Moran, T. J. Experimental aspiration pneumonia: inflammatory and reparative changes produced by intratracheal injections of autologous gastric juice and hydrochloric acid. (May-June) 579*
More, R. H. See Crowson, More, and King (May-June) 593*
——, **and Movat, H. Z.** Morphologic evidence of the hypersensitive pathogenesis of the lesions of experimental hypersensitivity and the collagen diseases. (May-June) 562*
Moritz, A. R., and Leuchtenberger, C. Systemic karyomegaly. Case report of a heretofore undescribed disease entity. (May-June) . . 564*
Movat, H. Z. See More and Movat (May-June) 562*
Muehrcke, R. C. See Pirani, Muehrcke, and Kark (May-June) . . . 594*
Muirhead, E. E.; Stirman, J. A., and Lesch, W. Reduction of postnephrectomy hypertension by homogenous renal transplant mediated by means other than desalting and dehydration. (May-June) . 585*
Mulligan, R. M. Hemangiopericytoma in the dog. (July-August) . . 773

Nadel, E. M., and Hilgar, A. G. Resistance to quadruple combination chemotherapy in leukemia L1210 in mice. (May-June) 605*
Newnum, R. L. See Gatzimos, Schulz, and Newnum (May-June, 567*; September-October) 791

Oester, Y. T.; Davis, O. F., and Friedman, B. Experimental arteriopathy: spontaneous, epinephrine-thyroxine, and cholesterol-induced forms. (July-August) 717
Ohringer, L. See Saphir and Ohringer (May-June) 575*
Olcott, C. T. Cell types and histologic patterns in carcinoma of the lung: observations on the significance of tumors containing more than one type of cell. (November-December) 975
Old, J. W.; Smith, W. W., and Grampa, G. Human lung in leukemia: observations on alveolar capillary leukostasis with reference to pathologic physiology. (May-June) 605*
Oliver, J. New approaches to the study of renal disorders. (May-June) . 592*
O'Neal, R. M., and Thomas, W. A. Rôle of pulmonary hypertension and thrombo-embolism in the production of pulmonary arteriosclerosis. (May-June) 578*

Orbison, J. L. See Schoenberg and Orbison (May-June) 573*
Orr, M. F., and McSwain, B. Tissue culture of human breast carcinoma. (January-February) 125
Ortega, L. G. See Mellors and Ortega (May-June) 604*

Pace, M. G. See Dammin, Goldman, Schroeder, and Pace (May-June) . 587*
Pani, K. C. See Tedeschi, Wagner, and Pani (May-June) 576*
Parkhurst, G. F., and Decker, J. P. Bacterial aortitis and mycotic aneurysm of the aorta: a report of twelve cases. (September-October) 821
Parrish, A. E. See Howe and Parrish (May-June) 594*, 603*
Parsons, E. H., Jr. See Dougherty, Saunders, and Parsons (May-June) . 475
Parthasarathy, M. See Popper, Clearkin, Kent, Parthasarathy, and Bruce (May-June) 594*
Pearce, J. M. Effect of hyaluronidase antagonists on the reaction of the host to viral infection. (May-June) 562*
Peters, E. R., and Farber, E. Histochemical localization of certain oxidative enzymes during the production of infarcts of the rat kidney. (May-June) 590*
Pickett, J. P. See Margolis and Pickett (May-June) 565*
Pirani, C. L.; Muehrcke, R. C., and Kark, R. M. Renal diseases as studied by serial kidney biopsy. (May-June) 594*
Plaut, A. Adrenal necrosis and thrombosis in routine necropsies. (January-February) 93
Playfair, F. D. See Donohue, Playfair, and Whitaker (May-June) . . 565*
Pollak, O. J., and Cruz, P. T. Serum elpherograms in experimental nephropathies of guinea-pigs. (May-June) 573*
Popper, H. See Elias and Popper (May-June) 596*
——; **Clearkin, K.; Kent, G.; Parthasarathy, M., and Bruce, C.** Coarse nodular cirrhosis produced by ethionine. (May-June) 594*
——, **and Elias, H.** Histogenesis of hepatic cirrhosis studied by the three-dimensional approach. (May-June) 405
Powell, C. S., and Swan, L. L. Leprosy: pathologic changes observed in fifty consecutive necropsies. (November-December) 1131
Pressman, D. See Mellors, Arias-Stella, Siegel, and Pressman (July-August) 687
Prior, J. T., and Hutter, R. V. P. Intimal repair of the aorta of the rabbit following experimental trauma. (January-February) 107

Race, G. J. See Larsh, Jeffries, and Race (May-June) 561*
Randall, C. C. Susceptibility *in vitro* of adult horse tissue to equine abortion virus: previously undescribed lesions. (November-December) 1165
Ratner, I. M. See Ehrlich and Ratner (November-December) . . . 1013
Reiner, L. See Russfield and Reiner (May-June) 572*
——. See Schlesinger and Reiner (May-June) 443
Rice, W. G. Qualitative analyses of antigens of myeloma serums with the agar-gel technique. (May-June) 574*
Riess, B. See Spain, Molomut, and Riess (May-June) 607*
Rigdon, R. H. Atypical cirrhosis in the duck produced by methylcholanthrene. (May-June) 461
——. Neurogenic tumors in the duck: an experimental study. (May-June) 583*
Rinehart, J. F. See Toreson and Rinehart (May-June) 569*
——; **Farquhar, M. G., and Gould, E.** Fine structure of the renal glomerulus. Histochemical and electron microscopic observations. (May-June) 587*
Roca-Garcia, M. See Love and Roca-Garcia (September-October) . . 901
Rogers, S. Influence of the intensity of exposure upon the number of pulmonary adenomas initiated by a single *in vitro* exposure to ultraviolet irradiation. (May-June) 582*

Roman, I. See Rosenthal, Finamore, Hunter, Hunter, and Roman (May-June) . . . 568*
Rosenthal, S. R.; Finamore, F.; Hunter F. R.; Hunter, A. S., and Roman, I. On the pathogenesis of death due to burning of the skin. (May-June) . . . 568*
Ruddle, F. See Berman, Stulberg, and Ruddle (May-June) . . . 580*
Russell, R. P., and Berthrong, M. Bacterial endocarditis produced by the tubercle bacillus. (May-June) . . . 577*
Russell, W. O. See Wynne and Russell (May-June) . . . 584*
——; **Guraieb, S.; Wiltberger, W. W., and Clark, R. L., Jr.** Pigment production and biologic behavior in 380 cases of malignant melanoma. (May-June) . . . 585*
Russfield, A. B., and Reiner, L. Possible endocrine significance of hypophyseal tumors in man. (May-June) . . . 572*

Saphir, O. Signet-ring cell carcinoma of the urinary bladder. (March-April) . . . 223
——, **and Ohringer, L.** Coronary arteriosclerotic heart disease in the younger age group; its greater frequency among an increasingly older necropsy population. (May-June) . . . 575*
Saunders, L. Z. See Dougherty, Saunders, and Parsons (May-June) . . . 475
Scherer, W. F., and Syverton, J. T. The viral range *in vitro* of a malignant human epithelial cell (strain HeLa, Gey). III. Studies with pseudolymphocytic choriomeningitis virus; general discussion. (January-February) . . . 31
Schlesinger, M. J., and Reiner, L. Focal myocytolysis of the heart. (May-June) . . . 443
Schlumberger, H. G. Transplantation of the parakeet pituitary tumor. (May-June) . . . 583*
Schoenberg, M. D., and Orbison, J. L. Factors influencing metachromasy in solutions. (May-June) . . . 573*
Schroeder, H. A. See Dammin, Goldman, Schroeder, and Pace (May-June) . . . 587*
Schulz, D. M. See Gatzimos, Schulz, and Newnum (May-June, 567*; September-October) . . . 791
Schwarz, J. See Adriano and Schwarz (September-October) . . . 859
——. See Straub and Schwarz (May-June) . . . 599*
——; **Silverman, F. N.; Straub, M.; Levine, S., and Adriano, S.** Relation of splenic calcification to histoplasmosis. (May-June) . . . 606*
Scott, E. B. Histopathology of amino acid deficiencies. IV. Tryptophan. (November-December) . . . 1111
Scotti, T. M. Basophilic degeneration of the myocardium. (May-June) . . . 606*
Shapiro, J. L.; Lux, J. J., and Sprofkin, B. E. Histoplasmosis of the central nervous system. (March-April) . . . 319
Shay, H. See Harris, Shay, and Gruenstein (March-April) . . . 367
Sheldon, W. H. See Bauer, Flanagan, and Sheldon (May-June) . . . 600*
Sherrick, J. C. See Laipply and Sherrick (May-June) . . . 599*
Siegel, M. See Mellors, Arias-Stella, Siegel, and Pressman (July-August) . . . 687
Silverman, F. N. See Schwarz, Silverman, Straub, Levine, and Adriano (May-June) . . . 606*
Smetana, H. F., and Cohen, B. M. Mortality in relation to histology in Hodgkin's disease. (May-June) . . . 597*
——, **and Johnson, F. B.** Neonatal jaundice with giant cell transformation of the hepatic parenchyma. (July-August) . . . 747
Smith, W. E. See McTurk, Eckardt, Smith, and Cooper (May-June) . . . 582*
Smith, W. W. See Old, Smith, and Grampa (May-June) . . . 605*
Sommers, S. C. Glomerular ultraviolet absorptions after cortisone therapy, simulating diabetes mellitus. (May-June)] . . . 570*
——. See Meissner and Sommers (May-June) . . . 571*

Spain, D. M.; Molomut, N., and Riess, B. Effect of audiogenic stimuli on wound healing in DBA/1 mice. (May-June) 607*
Sprofkin, B. E. See Shapiro, Lux, and Sprofkin (March-April) . . . 319
Stanton, M. F. See Wyatt, Sugarbaker, and Stanton (January-February) 143
Steer, A. Pathology of hemorrhagic fever. A comparison of the findings — 1951 and 1952. (March-April) 201
Steiner, P. E. See Elberg, Steiner, and Doll (November-December) . . 1065
Stembridge, V. A. See Brindley and Stembridge (May-June) 576*
Stern, H. See Kline, Bloomer, Stern, and Liebow (May-June) . . . 574*
Sternberg, W. H. Association of masculinizing ovarian hilus cell tumors, ovarian stromal hyperplasia, and lutein-like cell proliferation. (May-June) 571*
Stirman, J. A. See Muirhead, Stirman, and Lesch (May-June) 585*
Straub, M. See Schwarz, Silverman, Straub, Levine, and Adriano (May-June) 606*
——, and Schwarz, J. Healed primary complex in histoplasmosis. (May-June) 599*
Stuart, J. R. See Waugh and Stuart (May-June) 575*
Stulberg, C. S. See Berman, Stulberg, and Ruddle (May-June) . . . 580*
Stumpf, H. H. See Wilens and Stumpf (March-April) 275
——, and Wilens, S. L. Pancreatic lesions and peripancreatic fat necrosis in cortisone-treated rabbits. (May-June) 563*
Sugarbaker, E. D. See Wyatt, Sugarbaker, and Stanton (January-February) 143
Swan, L. L. See Powell and Swan (November-December) 1131
Swanton, M. C., and Brinkhous, K. M. Hemophilic arthropathy in dogs. (May-June) 596*
Syverton, J. T. See Scherer and Syverton (January-February) . . . 31

Tedeschi, C. G.; Wagner, B. M., and Pani, K. C. Clinical significance of the Aschoff body based on morphologic and histochemical observations. (May-June) 576*
Thal, A. Selective renal vasospasm and ischemic renal necrosis produced experimentally with staphylococcal toxin. Observations on the pathogenesis of bilateral cortical necrosis. (March-April) . . . 233
Thomas, L. See Brunson, Davis, and Thomas (July-August) . . . 669
——. See Brunson, Gamble, and Thomas (May-June) 489
——. See Brunson, Thomas, and Gamble (July-August) 655
Thomas, W. A. See O'Neal and Thomas (May-June) 578*
Thorn, G. W. See Dammin, Hume, Merrill, Miller, and Thorn (May-June) 586*
Timpe, A. See Vogel, Michael, and Timpe (May-June) 535
Toreson, W. E., and Rinehart, J. F. Histochemical observations on hyalinized islets of Langerhans in diabetes mellitus. (May-June) . 569*
Towbin, A. Basic pathologic forms of cerebral palsy. (May-June) . . 567*
Tullis, J. L.; Lamson, B. G., and Madden, S. C. Pathology of swine exposed to total body gamma radiation from an atomic bomb source. (January-February) 41

Van Patter, H. T., and Whittick, J. W. Heterotopic ossification in intestinal neoplasms. (January-February) 73
Villella, J. B. See Gould, Gomberg, Bethell, Villella, and Hertz (May-June, 561*; September-October) 933
Vogel, R. A.; Michael, M., Jr., and Timpe, A. Cortisone in experimental histoplasmosis. (May-June) 535
Volk, B. W. See Aronson, Volk, and Epstein (July-August) 609
——, and Goldner, M. G. Effect of large doses of growth hormone on the pancreatic alpha cells in the rat. (May-June) 569*

Wachstein, M., and Meisel, E. Histochemical localization of enzymatic activities in various portions of the mammalian nephron. (May-June) 588*

——, and ——. Succinic dehydrogenase activity in myocardial infarction and in induced myocardial necrosis. (March-April) 353
Wagner, B. M. Electrophoretic analysis of amyloid. (May-June) . . 573*
——. See Tedeschi, Wagner, and Pani (May-June) 576*
Warren, S. See Custer, Gunter, Warren, and Collier (May-June) . . . 597*
Wattenberg, L. W. Studies on rickettsial toxins. III. Histochemical survey of selected tissue enzymes in mice receiving murine typhus toxin. (September-October) 875
Waugh, D., and Stuart, J. R. Sodium and potassium content of rabbit plasma and erythrocytes in relation to age and to blood pressure. (May-June) 575*
Westrick, M. L. See Gross, Westrick, and McNerney (May-June) . . 579*
Whitaker, L. See Donohue, Playfair, and Whitaker (May-June) . . . 565*
Whittick, J. W. See Van Patter and Whittick (January-February) . . 73
Wilens, S. L. See Stumpf and Wilens (May-June) 563*
——, **and Stumpf, H. H.** Nodular and fatty glomerular lesions in rabbits on cortisone. (March-April) 275
Wiltberger, W. W. See Russell, Guraieb, Wiltberger, and Clark (May-June) 585*
Wissler, R. W. See Hopps and Wissler (March-April) 261
Wyatt, J. P.; Sugarbaker, E. D., and Stanton, M. F. Involvement of internal mammary lymph nodes in carcinoma of the breast: studies of the extended radical mastectomy. (January-February) . . . 143
Wynn, J. O. See Baker and Wynn (May-June) 599*
Wynne, E. S., and Russell, W. O. Pathogenesis of bovine ocular squamous cell carcinoma: histopathologic and clinical studies. (May-June) 584*

Yesner, R. Renal Shwartzman phenomenon in trichinosis. (May-June) 592*

Zimmerman, H. M. The nature of gliomas as revealed by animal experimentation. (January-February) 1
Zimmerman, L. E. See Anderson and Zimmerman (November-December) 1083